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# SOME PECULIARITIES IN THE GERMINATION OF BRUCELLA

By

J. B. Polding

## INTRODUCTION

From time to time previous workers have encountered abrupt breaks in the orderly germination of cultures seeded with serially diluted bacterial suspensions, such as in plate counts. The most notable descriptions of this phenomenon are probably those of Webster (1925) and Webster and Baudisch (1925) For the most part, however, such observations have received scant general interest, while, as far as the genus *Brucella* is concerned, no conscious record of such a break has been found throughout a very extensive literature. Nevertheless, whilst attempting to count *Brucella* in liver agar made at Mukteswar, the writer has encountered this phenomenon again and again. Since such an occurrence has not been widely commented on and as liver agar, before the advent of tryptose, has been considered the medium *par excellence* for the isolation of sparsely seeded *Brucella*, it has been assumed that various preparations of liver differ—some being normally fertile and others not.

It was considered, however, that conditions which support the germination of 100 million cells, whilst not permitting the growth of, say, 10 million, were sufficiently arresting to demand a more extensive investigation. This paper, therefore, is a condensed note of an examination of the phenomenon involved.

## METHODS

*Stock cultures.* Throughout the work stock cultures of *Brucella* were transplanted monthly on tryptose agar. When a strain was to be used for experiment, it was subcultured on a tryptose slant and incubated for 48 hours at optimum temperature. Type cultures were re-identified from time to time.

*Suspensions.* Suspensions were prepared by washing off a 48-hour culture in saline and adjusting the density of the washings to 5 on Brown's scale, whence the tenfold dilution series was prepared. Dilutions were used within an hour of preparation and the technique of Wilson *et al.* (1935) was followed throughout.

*Plating.* Plates were seeded by adding 1 ml. of a $10^{-n}$ dilution to plate n and then 15 ml. of the melted agar preparations were poured and the plate shaken. When separate additions, *e.g.*, liver extracts, were made to a medium, the required quantity was added to the plate at the time of seeding. The approximate theoretical cell density of plate 1 is $10^9$, of plate 2, $10^8$ and so on. After preparation, plates were inverted and incubated for five days at optimum temperature before final reading.

*Media.* Tryptose agar was made according to the maker's* instructions on the bottle. Except when a specific technique is described in the text;

* Difco Laboratories Inc. Baird and Tatlock, London.

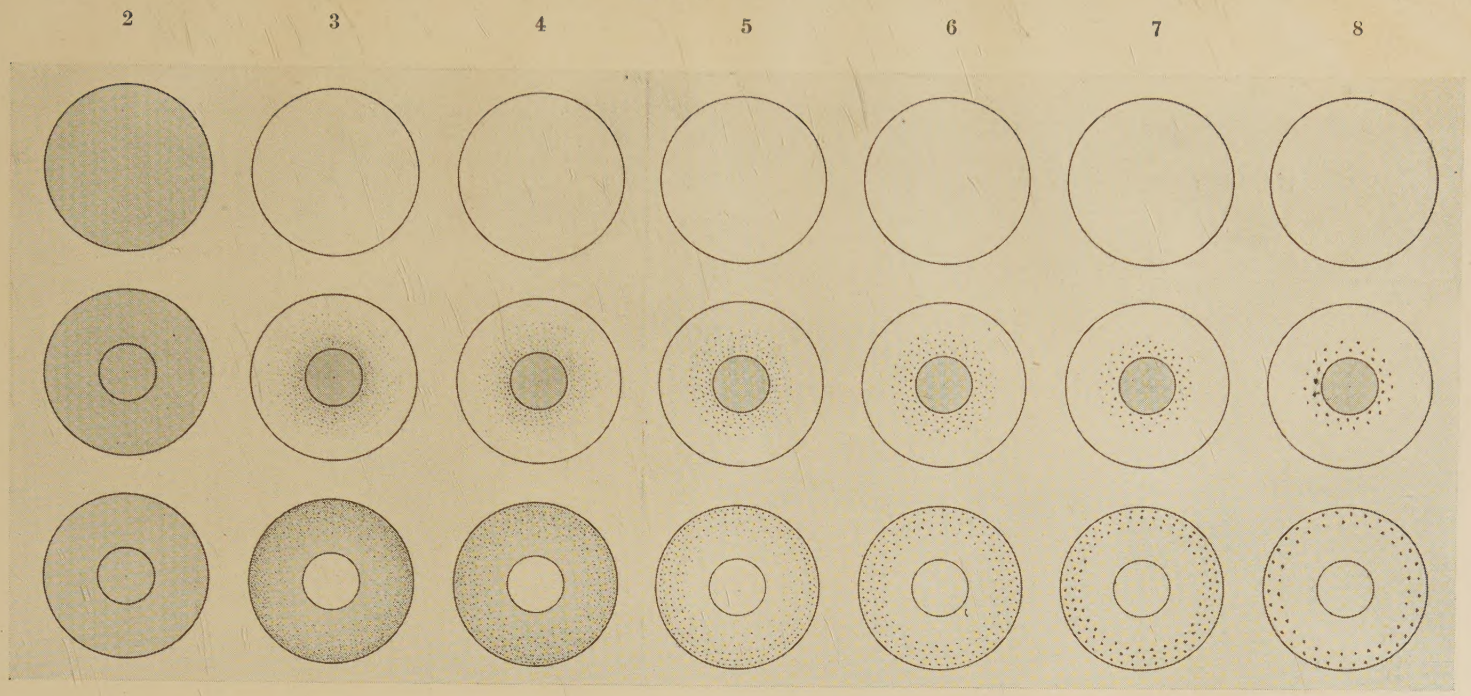
liver agar was prepared according to the method recommended by Huddleson (1939). The agar used was unwashed Japanese shred. Salt agar is the term applied to a medium consisting of Japanese agar 3·0 per cent. and sodium chloride 1·0 per cent. in distilled water. The solid medium bearing the reference "Maculloch's" was made by adding 3 per cent. of Japanese agar to Maculloch's and Dic's (1943) basal formula (used by them in fluid form). In the few instances where defibrinated blood additions are referred to as "heated" or "autoclaved" (Table 8, items 1 to 5), the medium was prepared in the manner of Levinthal (1918) and then autoclaved, or not, as the case might be. Wherever else additions are mentioned, the basal media were prepared seperately and autoclaved and the requisite addition made to the plate at the time of seeding. When coagulable blood enrichments were heated, they were diluted three times in normal saline, heated at 90° to 95°C for 15 to 20 minutes and the coagulum removed by centrifugation. The supernatant fluid was then added to the plates in thrice the volume of comparable unheated samples to compensate for the dilution made before heating.

*Liver extracts*. Concentrated liver extracts were made by adding 135 g. of minced liver to 150 ml. of distilled water (= 1 basal unit). The mixture after 24 hours in the refrigerator (0° to 5°C), was steamed for 1½ hours and then decanted on to a muslin cloth placed on a fine wire sieve, where it was stirred until most of the fluid had filtered off. The liver pulp and its previous container were then washed twice with 20 ml. of distilled water. The pulp was finally freed of all fluid in a fruit squeezer and discarded. All the fluid from these operations was collected and cleared by one or two filtrations through paper. Any further special processing such as fermentation or dialysis was introduced at this point. The infusion was then evaporated over boiling water until the volume was less than 40 ml. All deposits were resuspended in the fluid and the vessel washed twice with distilled water, the washings being added to the extract. After adjustment to *p*H 7·3 the extract was autoclaved, cleared by centrifugation and made up to an exact 40 ml. Two ml. added to 15 ml. of salt agar is equivalent, in terms of original weight of liver mince, to the concentration of mince extract in Huddleson's liver agar.

## CULTURAL MODIFICATIONS WHICH AFFECT GERMINATION

In other work (Polding, unpublished), 21 saline suspensions of *Brucella* (Str. 19) adjusted to a density between 5 and 6 on Brown's scale gave a mean viable count of $(108{\cdot}5 \pm 4{\cdot}18) \times 10^8$ organism per ml. (i.e., about double the haemocytometer count given in Table C of the Burroughs Wellcome Brown's tubes). These counts were made, using Wilson's technique of plating in solid medium (Wilson, 1935); but since no count could ever be obtained on the liver agar prepared at Mukteswar, tryptose agar was invariably used.

Whilst using tryptose the colonisation of the plates prepared from the *Brucella* dilutions $10^{-1}$ to $10^{-9}$ (designated plates 1 to 9) decreased in density from the first to the last plate, but, if identical dilutions were plated in liver agar, within certain limits of variation, growth was continuous in plates 1 and 2 (and sometimes 3) and ceased absolutely thereafter (plate 1, fig. 1). This cessation point in orderly




(Plates 1 & 9 omitted from each series)

FIG. 1.—Diagram of serial plate cultures Nos. 2 – 8 of *Brucella* in defective liver agar. Germination (grey shading) is total in plate 2 and nil in plates 3 and upwards.

FIG. 2.—Diagram of a similar series to that of Fig. 1 but with the plates containing a superimposed patch of densely seeded liver agar. The patches have germinated totally. In plates 3 and upwards there is a halo of satellisation round each patch.

FIG. 3.—Diagram of serial plate cultures of *Brucella* in 0·1 per cent. tryptose in agar with a superimposed patch of unseeded defective liver agar. A halo of infertility occurs round the patch in plate 3 and upwards.

Shading = Continuous growth

Dots = Colours slightly exaggerated

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germation will be denoted the "break"; the liver agar in which it occurs "defective." Further, if plate cultures of a closer bacterial dilution range be interpolated between a fully fertile plate 2 and an infertile plate 3, the germination will—apart from a few stray atypical groups of colonies—again show an abrupt break, *i.e.*, there will be no gradual tailing off of growth (Table 1).

TABLE I

THE END-POINT OF *Brucella* (STRAIN 19) GERMINATION IN CLOSE RANGE DILUTIONS OF BROWN'S SCALE 5 IN LIVER AGAR

| *Test* | *Dilutions of Brown's scale 5 plated* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1/40 | 1/60 | 1/80 | 1/100 | 1/200 | 1/400 | 1/600 | 1/800 | 1/1000 |
| Using washed cells | ++++ | ++++ | ++++ | ++++ | 2 cols. | 2 S.S. | 2 S.S. | 1 S.S. | nil |
| Using unwashed cells | ++++ | ++++ | ++++ | 20 cols. | 7 S.S. | ++++ | 7 S.S | 10 S.S. | nil |

In the tables, the decreasing pluses represent decreasing colonisation; cols. = colonies; c = contaminated; M = colonies only visible with the low power; L = colonies only visible with the hand-lens; S.S. = isolated satellitic system in an otherwise non-germinating plate. In tables and text, Str. 19 = Cottons strain 19; A.1, M.1 and S.1 or 3 = type specific *abortus, melitensis* and *suis* strains respectively. Counts up to 100 are correct, counts in round figures of 100 or above are estimates.

## THE EFFECTS ON THE BREAK OF VARYING THE METHOD OF PREPARATION OF LIVER CULTURES

### *Modifications of technique that do not affect the break*

In a preliminary series of experiments it was ascertained that the following modifications of technique did not materially affect the position of the break, *viz.*, (a) use of liver broth in place of liver agar; (b) pipetting of bacterial dilutions on to the surface of cold agar; (c) incubation of the plates in air, or in air with 5, 10, 20 or 40 per cent. $O_2$, or in air with 10, 20 or 40 per cent. $CO_2$; (d) use of unwashed or four times washed *Brucella* for making the suspensions; (e) preparation of the dilution series—and hence of the pipetted seedings—in 0·5, 2·0 or 5·0 Bacto-tryptose with normal saline, rather than in normal saline alone.

### *The effect of varying the pH of finished media*

Two experiments were made in which the final *p*H of the medium was varied. The effect upon the position of the break is shown in Table 2, which combines the experiments.

TABLE II

THE INFLUENCE OF *p*H CHANGES ON END-POINTS

| *p*H | *End-point in tryptose agar* | *p*H | *End-point in liver agar* | |
|---|---|---|---|---|
| 6·5 — 6·6 | $10^{-8}$ 54 cols. | 6·4 | $10^{-3}$ — | First experiment |
| 6·8 | $10^{-8}$ 106 cols. | 6·7 — 6·8 | $10^{-3}$ ++++ : $10^{-4}$ — | |
| 7·2 | $10^{-8}$ 166 cols. | 7·0 | $10^{-3}$ ++++ : $10^{-4}$ — | |
| 6·8 — 7·0 | $10^{-7}$ ++ | 6·6 | $10^{-3}$ ++++ : $10^{-4}$ — | Second experiment |
| | | 6·8 | $10^{-6}$ +++ : $10^{-7}$ — | |
| | | 7·0 | $10^{-5}$ +++ : $10^{-6}$ — | |
| | | 7·2 | $10^{-5}$ +++ : $10^{-6}$ — | |
| | | 7·4 | $10^{-5}$ +++ : $10^{-6}$ — | |

Strain 19 used.

The results indicate that *Brucella* was not very fastidious about *p*H in a non-defective medium; the liver medium of the first experiment was far more defective than that of the second; defectiveness is intensified at low *p*H; the optimum *p*H of defective media lies about the neutral point with greater safety on the alkaline side. In all subsequent experiments, therefore, the *p*H of finished media was made 7.2 to 7·4 before autoclaving.

*The effect of filtration*

Although Huddleson (1939) advises against the clarification of liver agar by filtration, at Mukteswar it is a common practice to filter solid media through Metasil in a Metafilter. It was on such liver agar that the phenomenon was originally noted and so as to discover if this was the cause of the break, the effect of filtration was examined in several experiments with similar results. Two experiments in which the response of four *Brucella* strains was tested are illustrated in Table 3. They show that of the four strains seeded, Str. 19 germinated least well, *abortus* (A.1) and *melitensis* (M.1) had similar and intermediate fertility, whilst *suis* (S.3) germinated most readily; the liver media of the first experiment were on the whole more defective than those of the second, though their preparation was in many respects comparable; filtration greatly increased the germinal value of the medium.

The relative fertility of the strains is typical and has been seen again and again throughout this work; its significance will be considered in the discussion. The variation in defectiveness between different batches of liver is also commonplace and the reason for this is of course linked with the basis of this enquiry. The improvement in the germinal value of liver after filtration is not only contrary to Huddleson's concepts but is perplexing and at this stage inexplicable.

In some further experiments liver infusion was separately mixed with (1) Metasil, (2) kiesulghur and (3) activated charcoal and stored in

TABLE III

THE RESPONSE OF FOUR *Brucella* STRAINS IN FILTERED AND UNFILTERED LIVER AGAR

| *Medium* | *End-point with* | | | |
|---|---|---|---|---|
| | *Str.* 19 | A.1 | M.1 | S.3 |
| 1st experiment : Filtered liver agar | $10^{-3}$ — | $10^{-6}$ + : $10^{-7}$ — | $10^{-4}$ +++ : $10^{-5}$ c : $10^{-6}$ — | $10^{-7}$ +± |
| Unfiltered liver agar | $10^{-3}$ — | $10^{-3}$ — | $10^{-3}$ — | $10^{-3}$ : $10^{-4}$— |
| 2nd experiment : Huddleson's liver agar (as prescribed by him) | $10^{-3}$ — | $10^{-4}$ +++ : $10^{-5}$ — | $10^{-4}$ +++ : $10^{-5}$ — | $10^{-7}$ 500 cols. |
| Mukteswar liver agar, unfiltered | $10^{-3}$ — | $10^{-5}$ ++ : $10^{-6}$ — | $10^{-5}$ ++ : $10^{-6}$ — | $10^{-7}$ 500 cols. |
| Mukteswar liver agar, filtered | $10^{-5}$ ± $10^{-6}$ — | $10^{-7}$ 500 cols. | $10^{-7}$ 500 cols. | $10^{-7}$ 500 cols. |
| Tryptose control (2 made) | $10^{-7}$ 500 cols. | $10^{-7}$ 500 cols. | $10^{-7}$ 500 cols. | $10^{-7}$ 500 cols. |

The agar was filtered through 5–10 g. of Metasil on paper in a Buchner funnel kept warm in a steamer for several hours.

the cold for several hours with occasional shaking. In one of these experiments subdivisions of such mixtures were brought to a *p*H of 6·0, 6·7, 7·2 and 8·4 respectively. After removal of the solids the infusions were concentrated by evaporation at 90° C and after the *p*H was adjusted to 7·4, were autoclaved. These concentrates were added to *Brucella* seeded silica pre-gels and plated. Although a slight improvement in the germinal value of the preparations treated with Metasil was noted, its significance was not considered great enough to warrant a continuation of work on absorption.

*Heating Effects*

The effect of heating liver preparations at different temperature levels and for varying periods was examined in three experiments.

In the first, some ox-liver agar, which in another experiment had proved to be non-germinal in plate 3 for Str. 19, was selected for further heat treatment. This medium had already been heated for 1 hour and 50 minutes in the infusion stage, warmed during the melting of the agar and once autoclaved. All that remained was now melted and divided into four lots. Medium A was brought to *p*H 7·4 and stored in the cold. Media B, C and D were heated for six hours at 60° C, 90-95° C and 107° C respectively. After adjusting their *p*H to 7·4 they were autoclaved together with medium A and finally remelted for pouring into the plates. The results of this experiment suggest (Table 4) that a certain degree of heating improves a poor medium, even after all but a trace of coagulum

TABLE IV

EFFECT OF VARYING TEMPERATURE AND TIME OF HEATING

| *Medium* | *Dilutions of seed plated* | | | | | |
|---|---|---|---|---|---|---|
| | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
| 1. The original medium before further heating (separate experiment) | S.S. | S.S. | — | — | — | — |
| 2. Medium of 1, remelted *p*H checked, reautoclaved (called Medium A) | ++++ | +++± | +++ | +++ (late) | —M | — |
| 3. Medium of 1, remelted, heated at 60° C. for six hours, *p*H checked, reautoclaved (called Medium B) | ++++ | ++++ | ++± | —M | — | — |
| 4. Medium of 1, remelted, heated at 90–95° C. for six hours, *p*H checked, reautoclaved (called Medium C) | ++++ | —M | — | — | — | — |
| 5. Medium of 1, remelted, heated at 107° C. for six hours, *p*H checked, reautoclaved (called Medium D) | ++++ | —M | — | — | — | — |

Strain 19 used.

has been removed. On the other hand, heating beyond a certain point is clearly deleterious.

It is to be noted, however, that observations made much later indicate that, in concentrated extracts at least, defectiveness disappears during storage. It is likely, therefore, that this liver agar had spontaneously become germinal during several days' storage between the first test and the preparation of media A to D. If this is so, then prolonged additional heating merely has the effect of increasing defectiveness.

In the second experiment (Table 5) the effect of long and short heating at 90° to 93° C. of the infusion at different *p*H was examined. After the infusion stage, all media lots were converted to liver agar using Huddleson's technique. In this experiment S.3 germinated totally by the third day of incubation, so the results of the experiment are to be seen in the germination of Str. 19 only. They show

TABLE V

THE EFFECT OF VARIOUSLY HEATING

| *Hours heated* | p*H at 93° C. (2nd experiment)* | | |
|---|---|---|---|
| | 6·0 | 7·0 | 7·4 |
| 1 | $10^{-7}$ 12 cols. | $10^{-6}$ 35 cols. : $10^{-7}$ — | $10^{-7}$ 27 cols. |
| 6 | $10^{-2}$ +++± : $10^{-3}$ — | $10^{-3}$ +++± : $10^{-4}$ — | $10^{-2}$ +++± : $10^{-3}$ — |
| Tryptose control | | $10^{-7}$ 500 cols. | |

Results are from Strain 19. S.3 plated in parallel germinated totally.

that one hour's heating in alkaline reaction gave the best germination, whilst six hours' heating was in all cases deleterious. The overall deduction is that short rather than long heating is optimal for growth, whilst long heating appears to enhance defectiveness.

The third experiment was primarily a test of the germinal value of two different livers, but the first liver was heated in a variety of ways. This liver was taken from a rinderpest-fevered bull killed on the fifth day of the disease. The organ weighed 4 lb. (average 3½ lb.), it was pale brown and on mincing was sloppy and comparatively bloodless. After mincing, six equal lots of mince (A to F) were weighed and added to distilled water at the rate of 1 lb. per 500 ml. All were stored in the cold for 24 hours. Lots A, C and E were then heated for one hour, and lots B, D and F for three hours at 50-55°C, 70-75°C and 90-95° C respectively. The *p*H of all samples was made 7·6 before heating. The second liver was taken from a hill bull that had died of debility. It weighed 2 lb., was deep maroon in colour and contained much blood. It was made into lot G exactly as E. Lots A, B, C, and D coagulated incompletely after heating and passed through a fine wire sieve in the form of a porridge. Although heated side by side with E, preparation G was the best coagulated of all samples; its colour, moreover, was the dark greenish brown usually associated with liver infusion. All infusions were now converted to liver agar using Huddleson's technique and tubed. After autoclaving, there was considerable coagulum in the tubes of the less heated samples, but care was exercised to avoid transferring much of this deposit to the plates. When, however, one or two coagula inadvertently passed into a plate a halo of germination appeared around some of them in an otherwise non-germinating plate.

In this experiment the over-all value of the medium was less than that of the last one, for here S.3 partially germinated in the best preparations only, while Str. 19 failed to germinate at all. The results (Table 5) were, nevertheless, very interesting. In the first place, the liver of the dead hill bull (sample G) gave no germination in plate 2

LIVER INFUSIONS AT DIFFERENT *p*H

| *Hours heated* | *Temp. at* pH 7·6 (*3rd experiment*) | | |
|---|---|---|---|
| | 50–55° C. | 70–75° C. | 90–95° C. |
| 1 (1st liver) | $10^{-2}$ : $10^{-3}$ — M<br>A | $10^{-3}$ +++ : $10^{-4}$ — M<br>C | $10^{-7}$ few cols.<br>E |
| 1 (2nd liver) | | | $10^{-2}$ — M<br>G |
| 3 (1st liver) | $10^{-5}$ + : $10^{-6}$ — M<br>B | $10^{-6}$ few cols. : $10^{-7}$ —<br>D | $10^{-2}$ ++++ : $10^{-3}$ —M<br>F |
| Tryptose control | | | $10^{-7}$ 150 cols. |

Results are from S.3. Strain 19 plated in parallel failed to germinate except in the control.

with either Cotton's strain or with S.3, while the similarly prepared sample E of the rinderpest liver gave the best germination of all. In view of future findings it is to be noted that the liver of G contained the most blood. This result clearly illustrates how the germinal value of preparations made in an identical way from the liver of animals of the same breed, but in different condition, may vary. It may be added that the liver of the dead hill bull was stored in the cold for a shorter period than that from the rinderpest bull (24 hours as opposed to 48), but it had lain a few hours longer in the carcase before removal. Of the other results, heating the infusion at 90-95° C for one hour or at 70-75° C for three hours produced the most germinal media; heating at 90-95°C for three hours or at 50-55°C for one hour, gave the least. In a few further experiments it was confirmed that *p*H variations during extraction seem to exert little significance except that extreme acidity in the infusion stage gave a bad germinal preparation.

The foregoing experiments were regarded as preliminaries. They tend to demonstrate that apart from variations in the properties of the liver itself, which are clearly great, the only important extraction factor (of those examined) is heat. However, although the last experiment indicates that, for the particular liver used, the optimum period of steaming is about one hour, there is nothing at all to show that this datum holds for all livers. The over-all indication is that livers vary considerably in what might be called their potential defectiveness, and it is reasonable to suppose a potentially strongly defective liver might require less heating to produce a medium of given defectiveness than a potentially weak one.

*The effect of dialysis and ultrafiltration*

In the ensuing work liver infusions were prepared in the normal way (Huddleson, 1939) and were dialysed or ultrafiltered through collodian sacs (Duckworth, 1935). They were then concentrated by evaporation at 90°C in the manner described for preparing liver extracts on page 216. Such extracts were plated either in pre-gels, or in agar, seeded with a dilution series of *Brucella,* and the resultant growth noted.

In the first experiment, two unit volumes, A and B, each of 225 ml. of liver infusion were taken. Volume A was kept in the cold store as a control, and Volume B placed in the reservoir of an ultrafilter (Duckworth, 1935). The infusion was drawn through the sacs by negative pressure until the original volume of infusion had passed through, the reservoir being topped up with distilled water. The period of filtration was much longer than anticipated (about 18 hours) and as the work was done at room temperature the fluids on both sides of the filter sacs fermented; nevertheless the experiment was carried to its conclusion. The volume that remained in the sacs (Volume C) was made up to 225 ml. with distilled water. Volumes

A, B and C were then evaporated in parallel in the standard way and the extracts plated in *Brucella* seeded pre-gels (*p*H 7·0-7·4). The germination of these cultures are shown in Table 6.

This experiment demonstrates that fermentation rendered a defective infusion germinal; the fermented residue was slightly more germinal than the filtrate, and the germinal factor of the fermented products was cancelled by a factor in the unfermented extract. It is also to be noted that in this experiment, which was carried out during the monsoon, a considerable number of the gel cultures were contaminated; to this extent gel-plating is unsatisfactory at present.

The filtration experiment was repeated operating the filter in the refrigerator at 0°C, but the process was continued not only until the volume of infusion containing 1 unit had passed the sacs, but until most of the residue in the sacs became colourless (228 ml. of infusion were put into the reservoir and 308 ml. of filtrate were collected). The process took about 40 hours and no fermentation was noted. It was not possible to get all the coloured material through the membranes as a stagnant pocket of reddish fluid collected in the tip of each sac, but the bulk of the fluid in the sacs was colourless and cloudy. The ultrafiltrate was pale yellow and clear and gave a doubtful reaction for protein.

To confirm the fermentation effect of the last experiment, a portion of the unfiltered infusion control was allowed to ferment and tested for its germinal value, while the experiment was rounded off by including a sample of dialysed infusion. About 100 ml. of infusion was placed in Duckworth's sacs and dialysed for 24 hours at ca O°C against frequent changes of large volumes of tap water. This and the other samples were proportionately evaporated as usual and plated in *Brucella* seeded pre-gels.

The results (Table 6) confirm that the spontaneous fermentation of a potentially defective infusion renders it protentially germinal. They also suggest that, while the factor of defectiveness is non-diaylsable, it can be forced through a distended collodian sac. This may indicate that its molecular weight is more, but probably not much more, than about 2,000. It is, of course, likely that the growth factors of liver are dialysable and that the growth noted in C is the result of bacterial synthesis from a non-ultrafilterable pre-growth-factor or of the liberation of fresh growth factor during the heating subsequent to dialysis.

The cancelling effect of mixtures A and B, A and C and B and C conform with the general trend of the research. A third test of the inhibiting power of dialysed infusions not only confirmed the previous results, but the dialysis residue was shown to cancel the germinal value of 0·1 per cent. tryptose in agar.

It is appreciated, of course, that the foregoing observations are neither complete nor comprehensive; for instance, the disappearance of defectiveness as a result of filtration is by no means explained.

TABLE VI

THE EFFECTS OF DIALYSIS AND PRESSURE ULTRAFILTRATION OF LIVER INFUSIONS (TWO EXPERIMENTS)

| *Fraction of infusion* | *Seed* | | *Fraction of infusion* | *Seed* | |
|---|---|---|---|---|---|
| | *A.*1 | *Str.* 19 | | *A.*1 | *Str.* 19 |
| A. Control unfiltered | $10^{-2}$ — M | $10^{-2}$ — M | A. Control unfiltered | $10^{-3}$ — M | $10^{-2}$ — M |
| B. Filtrate fermented | $10^{-6}$ ± | $10^{-2}$ — M | B. Filtrate | $10^{-3}$ +++ : $10^{-4}$ — | $10^{-2}$ c : $10^{-3}$ — |
| C. Residue fermented | $10^{-6}$ ± | $10^{-3}$ +++ | C. Residue | $10^{-6}$ ± | $10^{-5}$ ++ : $10^{-6}$ not done |
| D. Fractions A and B | $10^{-2}$ — M | $10^{-2}$ — M | D. Fractions A and B | $10^{-3}$ — M | $10^{-2}$ — M |
| E. Fractions A and C | $10^{-2}$ — M | $10^{-2}$ — M | E. Fractions A and C | $10^{-3}$ c : $10^{-4}$ — M | $10^{-2}$ — M |
| | | | F. Fractions B and C | Not done | $10^{-2}$ — M |
| | | | G. Dialysis residue | $10^{-3}$ — M | $10^{-2}$ — M |
| | | | H. Control unfiltered but fermented | $10^{-6}$ ± | $10^{-2}$ +++± : $10^{-3}$ — M |

They do demonstrate, however, that during the hydrolysis of liver a non-dialysable *Brucella* inhibitor is released after a certain period of heating, the various type-specific strains respond variously to this inhibitor and livers taken from animals that are variously debilitated differ in what might be termed their inhibitor potential.

## EXPERIMENTS DEMONSTRATING SCME FUNCTIONS OF CELLS AND CELL PRODUCTS IN DEFECTIVE CULTURES

The findings of the previous section indicate that during the hydrolysis of liver a factor is produced which inhibits the germination of sparsely seeded *Brucella*. It appears to be non-dialysable but ultra-filtrable and its occurrence depends partly on the nature of the liver itself and partly on the degree of heat applied to the liver during hydrolysis. Further, the factor seems to be separable from the germinal factions of liver and when it is re-combined with them or with other nutrient media, such as 0·1 per cent. tryptose, it prevents germination.

But the mere demonstration of a *Brucella* inhibitor in liver does not fully explain the abruptness of the break phenomenon; for it is inconceivable that in random preparations of liver there is always just sufficient inhibitor to check the growth of $10^n$ cells in plate x, whilst there is insufficient to have any apparent effect on the germination of $10^{n-1}$ cells in the preceding plate. On some occasions, at least, partial growth could be expected in either plate x or plate $x^{-1}$ or in some plate of a close range dilution series interpolated between them. As, however, such a tailing off of growth has never been

observed, at any rate where seedings are dense, some further concept is needed to explain the phenomenon. In the current section an attempt has been made to discover the mechanism of this "all or nothing" type of germination and in order to do so it seemed desirable to examine the action of cell products and of the cells themselves in typically defective cultures.

As has already been noted in Table 1 and in other experiments (p. 217) the use of washed or unwashed cells seems to have no effect on the break. The unimportance of cell washings is emphasised by the observations that the break phenomenon is totally unaffected by the addition to defective cultures of 10 per cent. concentrations of Seitz filtrates of 15-day-old *Brucella* cultures grown in tryptose or liver broth and 10 per cent. concentrations of a Seitz filtrate of the washings of a 48-hour-old culture of *Brucella* grown on Roux flasks of liver agar. The possibility that Seitz filters may absorb factors from the broth must not be overlooked.

In two other experiments the break was similarly shown to be unaffected by the addition to the serially diluted live *Brucella* seed in defective liver agar of *Brucella* killed by heating at 55°C for one hour, or live $CO_2$ sensitive *Brucella* incubated in air and therefore non-germinal and added in such numbers as to bring up the total live and dead, $CO_2$ sensitive, cell population in plates 2 to 8 to the theoretical population of plate 1 of the series.

It is probable, therefore, that there are no diffusible stable cell products being carried over on the seed or with seeding fluids which interact with the inhibitor to produce a sharp break. If, therefore, the presence of neither cell washings, nor dead, nor live non-germinating cells affects the phenomenon, then, apart from cell extracts, there remains but the function of the live germinating cells themselves to examine. In order to test the influence of germinating *Brucella* cells on the non-germinating population of defective cultures, the following experiment was devised and repeated frequently with consistent results.

The tenfold *Brucella* dilutions prepared from a parent suspension were seeded in plates 1 to 8 as usual and defective liver agar was melted, poured and shaken in the normal manner. The series was duplicated, the second sample being set aside as a control. A further quantity of the same defective liver was now melted and seeded with the parent suspension of *Brucella* so as to make the seed density $10^{-1} \times 2$. This apar was then rolled and small quantities of it were poured on to the top of the set agar of plates 1 to 8 of the principal series so as to form a raised patch about 3 cm. dia. in the centre of the plate. When this patch in turn had set then these plates and the controls were inverted and incubated for five days. During incubation the control series germinated as usual in plates 1 and 2 alone (plate 1, fig. 1). In the principal series, on the other hand, the heavily seeded superimposed patch germinated totally and uniformly;

the subadjacent medium of plates 1 and 2 germinated as in the controls while, unlike the controls, in plate 3 and upwards the subadjacent medium was thickly populated with colonies which formed a halo 2 or 3 cm. deep around the patch. On the whole, colonies were largest near to the patch and smallest remote from it, whilst there was not even microscopic germination at the periphery. There was also, of course, colonisation in the shadow of the patch (plate 1, fig. 2). Str. 19 was used in this test, which was repeated, and other strains were similarly tested. After several trials it was seen that when Str. 19 was seeded in the patch and the subadjacent medium (called the bed) the halo in the more heavily seeded plates was usually restricted to a depth of 1 to 2 cm.; when A.1 or M.1 occupied both patch and bed, the halo usually spread half way to the edge, whilst when S.1 was used it was not uncommon for germination to be total right up to the periphery of the plate. The above reactions are, of course, examples of mass satellitism whereby infertile cells germinate under the influence of the richly fertile, densely seeded patch. It also appears that S.1 is most actively satellitic and Str. 19 least so.

It should be interpolated here that the writer was led to make the preceding type of experiment by the following accidental observation. During the monsoon the process of plating is gravely hindered at Mukteswar by constant contamination with a *B. subtilis*-like organism. Very frequently, therefore, a patch of *subtilis* was found interposed between the bottom of the plate and the medium Such patches did not generally spread much and were usually about 2-4 cm. dia. by the 4th day of incubation. Now it was noticed again and again that opposite such a patch (*i.e.,* in its shadow) *Brucella* germinated in an otherwise infertile plate. It is clear that here again *B. subtilis* is satellising the non-germination *Brucella* seed. That it does so is, at first sight, rather surprising, in view of the well recognised antibiotic properties of *subtilis*. However,, the identity of the satellite colonies was checked too often to admit of any mistake and, apart from calling attention to the point, all that can be added is that *Brucella* and *subtilis* are both obligatory aerobes, and it might not be impossible for two such genera to have respiratory functions in common.

In the next experiment the technique was reversed, *viz.,* a tenfold dilution series was plated as usual, but the medium of the beds consisted of 0·1 per cent. tryptose in salt agar, whilst the patches were formed solely of unseeded defective liver agar. In this case the control series of *Brucella* seedings in the uncomplicated plates germinated throughout the range, albeit belatedly and with small colonies. In the principal series, on the other hand, germination occurred similarly at the periphery of the plate, but the area around the patch remained clear of growth. During the fifth day of incubation this area tended to disappear by a gradual increeping of germina-

tion, especially at the densely seeded end of the series (plate 1, fig. 4).

It appears from the foregoing experiments that germination in defective liver agar is influenced by a factor—almost certainly a soluble and diffusable one—that is liberated by actively dividing *Brucella* (or *subtilis*) cells. It may be objected, of course, that if as a result of germination a diffusible fertility-factor is liberated into the substrate, then this factor should be present in the cell washings and cell additions used in the first five experiments of this section. The objection, however, is not quite watertight for there is no necessity whatever to suppose that this factor is stable or can operate for long after being separated from the source that generates it. An experiment was made, therefore, to try to demonstrate that this factor is indeed unstable. It has already been observed that the break phenomenon occurs in liver broth as well as in agar. It should be added that in broth the break usually occurs (with Str. 19) at about tube 5 or 6 of the tenfold series. Non-germinating cultures in liver both should, therefore, be prepared in dilution $10^{-6}$ and upwards.

For this experiment Berkfeld N filter candles were inserted into the neck of 250 ml. flasks so that their apices reached nearly to the bottom. The space between the neck of the flask and the candle and the entrance to the candle itself were plugged with wool and the whole set-up was sterilised. A measured volume of defective liver broth was introduced into each flask so as to immerse the greater part of the candle. The flasks were then incubated to ensure that they were sterile. Flask A was now seeded outside the candle with Str. 19 in the non-germinating dilution of $10^{-6}$. The inside of this candle was seeded heavily with S.1. Flask B was seeded inside the candle only with a heavy seed of S.1, Flask C was seeded on the outside of the candle only with a $10^{-6}$ dilution of Str. 19. After three days' incubation flask A showed germination outside the candle and *Brucella* were recovered and identified from this outer broth. Flasks B and C showed no germination outside the candle. The infertility of the outer broth of flash C showed that a $10^{-6}$ seeding in this particular broth was non-germinal so long as it received no influence from inside the candle. The germination in flask A, assuming that the candle was intact, suggests that the richly fertile S.1 inside the candle had induced germination in the outer culture, and in view of the satellitism noted in solid cultures this was not surprising. The sterility of the outer unseeded medium of flask B shows that significant amounts of the internal seed did not pass through the candle during the period of incubation. At the same time this broth must presumably have been the recipient of diffusible substances from the inner culture in the same way as that of flask A, where germination was induced. This broth was, therefore, pipetted off and 9 ml. volumes of it were distributed into tubes in which a tenfold dilution series of Str. 19 was prepared. A

sample of the original broth that had had no treatment was similarly disposed and likewise seeded, as a control. The two series thus prepared were then incubated for five days, when it was observed that the break occurred in the control and principal series alike at dilution $10^{-5}$. In other words, the broth of flask B had not been permanently activated.

Regrettably, circumstances did not permit the repetition of this experiment, and although the flask B part of it was done in triplicate, the results must be regarded as inconclusive. The tentative inference may be made, however, that the factor responsible for the satellisation of non-germinating *Brucella* cells is either very unstable or cannot operate when removed from its source of generation, while such a concept is at least in keeping with the other results of the section.

It is, of course, only reasonable to speculate as to what happens to the seed of a defective system if it remains in contact with the inhibitor for long, and it seems probable that it dies during five days' incubation. Thus, the agar of the first ungerminated plate of three defective systems was removed and chopped and each was added to 50 ml. of 2·0 per cent. tryptose broth. No germination occurred in any of these broths after five days' incubation and, as previous trials had shown that 14 ml. of 2·0 per cent. tryptose is more than enough to cancel an ordinary plate-quantity of inhibitor, the use of 50 ml. of trytose renders cancellation a virtual certainty. The action of inhibitor is, therefore, bactericidal and as, unlike penicillin, it is derived from the host in which the bacteria thrive, its function in immunity, or as a curative agent or attenuator of *Brucella* for vaccines may prove of considerable interest.

### NUTRIENT ADDITIONS WHICH MODIFY THE POSITION OF THE BREAK

In the preceding sections an attempt has been made to demonstrate the mechanism of the "all or nothing" germination of the break and two components appear to be concerned, *i.e.*, the presence of a bacterial inhibitor in the medium and the faculty of the germinating bacteria to synthesise a factor which cancels this inhibitor. It follows, therefore, that if substances can be discovered, which when added to a defective series shift the point of break to the right or abolish it altogether, then such substances are either closely allied with, or can substitute for, the postulated inhibitor-cancelling-factor, without necessarily being identical with it. The present section records the results that have been obtained from adding various substances to defective series with the view of altering the position of the break.

#### *Some examples of breaks*

Some examples of breaks with *Brucella* and one or two other genera on various media together with failures to get a break are shown in Table 7.

TABLE VII

SOME EXAMPLES OF "BREAKS"

| *Seed* | *Medium* | *Dilutions of seed plated* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ |
| *Salmonella* | Beef agar | | ++++ | +++± | +++ | ++± | ++ | c |
| *Pasteurella* | ,, | | +++± | — | — | — | — | — |
| *Streptococci* | ,, | +++M | few cols. | — | — | — | — | — |
| *Brucella* (Str. S.3) | ,, | ++++ | ++++ | +++± | +++ | — | — | — |
| ,, (Str. 19) | Potato agar* | +++ | — | — | — | — | — | — |
| ,, ,, | Liver agar | ++++ | ++++ | — | — | — | — | — |
| ,, ,, | Chocolate agar | ++++ | ++++ | +++± | +++ | ++± | | |
| ,, ,, | Chocolate agar, autoclaved | ++++ | ++++ | +++± | +++ | ++± | | |
| ,, ,, | Levinthal's agar, autoclaved | ++++ | ++++ | +++± | +++ | ++± | | |
| ,, ,, | Tryptose agar | ++++ | ++++ | +++± | +++ | ++± | ++ | +± |

* Mitchell and Moore (1941).

Although one or two other media were tried, a break could not be obtained with the strain of *Salmonella* used, for it seemed able to germinate fully on most simple media or not at all on non-nutrient agars. The break with *Pasteurella* looks "true" although it has not been confirmed by a demonstration of satellitism, while the break with *Streptococci* may or may not have been genuine. The foregoing observations have been included merely because they arouse interest. The results with *Brucella* have been confirmed by satellitism, except in the case of potato agar. The absence of a break in *Brucella* germination on blood media suggests that blood might contain inhibitor-cancelling-factors.

*Fractions of blood which modify the break in liver agar*

Before proceeding to the addition of blood fractions to liver agar, it seemed desirable to note the response of *Brucella* to the several common substances usually incorporated in empirical media.

The results of this work indicated, as far as *Brucella* are concerned, that the relatively pure Japanese agar used throughout the experiments was non-germinal in the presence of salt; a very crude Indian-made agar, with salts, was partially nutrient; glucose, with Japanese agar and salt, was non-nutrient, but with ammonium sulphate added, the medium became partially nutrient; Maculloch's salts and glucose were also partially nutrient: peptone (and it may be added casein hydrolysate) in salt agar was found to be nutrient, albeit deficiently. The term "deficient" is used here to mean that while the culture series germinates without a break, in the sense used throughout this paper, the individual colonies, though present in about the correct density, are reduced in size and appear late during the period of incubation. In a typically deficient series the colonies in plates 7 and 8 are visible to the naked eye, those in 8 being larger and, of course,

fewer than those in 7. In plate 6 the colonies can only be distinguished with the aid of the hand lens (L in the tables), while in plate 5 there may be uncertainty even with the lens. As the population increases in each lower plate in turn, it will become increasingly difficult to identify colonies even with the low power of the microscope (M in the tables), whilst in about plate 3 or 2 colonies cannot be distinguished even with this aid. This picture appears to be the logical outcome of a simple deficiency of food factors, *i.e.,* as the population increases there are less and less factors available per cell, so cells going short produce smaller and smaller colonies. It is manifestly a very different occurrence from the break form of germination, which cannot be a simple deficiency.

The slight nutrient effect of ammonium sulphate and Maculloch's salts is perplexing and is a point remaining for future explanation. Perhaps the Japanese agar, which could by no means have been regarded as vitamin-free, contained subminimal amounts of nutrients and the salts are "sparing" in action. It is to be noted that Huddleson's (1939) liver agar contains peptone, so it must be inferred from the defectiveness of many liver agars that the growth factors of peptone are neutralised by the inhibitor of liver.

The effect of adding various blood fractions to liver agar was now tried and the results (Table 8) show that there are two fractions capable of overcoming the inhibitor of liver, *i.e.,* a heatstable portion in coagulum-free, heated, defibrinated blood and a heatlabile one in washed, laked R.B.C. Both fractions, moreover, had to be added in about five per cent. concentration to be effective. That this is so, does not necessarily mean that the cancelling substance is not a trace-factor, for this substance itself may only be sparingly present in blood. It is evident that autoclaved milk also contains traces of a cancelling substance. It is to be noted that in this experiment citrated plasma failed to give growth, a result which must not be regarded as conflicting with Huddleson, Wood and Cressman's (1945) observation that the plasma of healthy bovines permits the growth of *Brucella,* while the plasma of *Brucella*-immune cattle does not, for Huddleson added plasma to tryptose and the growth he observed may have been wholly or in part due to the tryptose. Huddleson in fact is noting a tryptose-neutralising and not a growth-promoting action of plasma. In this experiment plasma was added to strongly inhibiting liver agar and reasonably enough gave no growth; nevertheless, as shown in the next section, plasma is partially germinal.

*The fractions of blood that enhance germination in deficient media*

Similar blood fractions were now added to Maculloch and Dic's (1943) basal salts in agar, a medium which has already been shown to be deficient for *Brucella.* As might be expected, the results (Table 8) show that the blood fractions which cancellel the inhibitor of liver, are those which enrich a deficient medium, and it is now seen that, in the absence of inhibitor, plasma is able to promote growth.

TABLE VIII

THE FRACTIONS OF BLOOD WHICH AFFECT GERMINATION IN DEFECTIVE AND DEFICIENT MEDIA

| *Blood fractions (bovine)* | *Defective liver agar* | *Deficient Maculloch's agar* |
|---|---|---|
| 1. Defibrinated, autoclaved whole blood. 1·0% | $10^{-3} \pm : 10^{-4}$— | |
| 2. Defibrinated, autoclaved whole blood. 5·0% | $10^{-7} \pm : 10^{-8}$ — | |
| 3. Defibrinated, heated whole blood. 5·0% | $10^{-8} \pm$ | $10^{-7}$ few cols. |
| 4. Serum, unheated. 5·0% | $10^{-3} +++\pm : 10^{-4}$ — | |
| 5. Serum, autoclaved. 5·0% | $10^{-3}$ — | |
| 6. Milk, autoclaved. 10·0% | $10^{-5} \pm : 10^{-6}$ c. : $10^{-7}$ — | |
| 7. Haemoglobin. 10·0% | $10^{-3}$ — | |
| 8. Washed R.B.C. laked, unheated. 0·5% | $10^{-3}$ — | |
| 9. Washed R.B.C. laked, unheated. 5·0% | $10^{-8} +$ | $10^{-6} + : 10^{-7}$ c. |
| 10. Washed R.B.C. laked, heated. 5·0% | $10^{-3}$ — | $10^{-3}$ |
| 11. Plasma of R.B.C. (citrated). 5·0% | $10^{-3}$ — | $10^{-5} + : 10^{-6} \pm 10^{-7}$ c. |
| 12. Addition 10 and 11 combined. 5·0% | $10^{-3}$ — | |
| 13. Heated, citrated whole blood. 5·0% | | $10^{-7}$ few cols. |
| 14. Liver agar control | $10^{-3}$ — | |
| 15. McCulloch's agar control | | $10^{-3}$ — |
| 16. Tryptose control | $10^{-7} +\pm$ | $10^{-7} +\pm$ |

Strain 19 used. R.B.C. washed five times. Heated enrichments coagulum-free. Plasma of R.B.C. was citrated throughout but oxalation was tried with similar results. Similar samples of rabbits' blood were tried in some cases with similar results.

By careful standardisation it ought to be possible to demonstrate that less blood additions are required to get maximal growth in a non-nutrient medium than in an inhibitory medium, but, at the time these experiments were made, insufficient was known about the phenomenon to demonstrate that this was so.

*A comparison of the cancelling value of blood fractions and vitamins*

Koser, Breslove and Dofman (1941) and Maculloch and Dic (1943) showed that biotin, nicotinic acid, calcium panthothenate, and thiamin are essential for the growth of some, but not all, strains of *Brucella*. A further experiment was, therefore, undertaken in which the action of these vitamins,* added each at the rate of 0·4 $\mu$g. per ml. of medium, was compared with that of the active blood fractions in McCulloch's agar. As, however, biotin was not available at the time and as *suis* strains alone are said not to require it, a *suis* type specific

* The vitamins were the generous gifts of La Roche Products, Ltd. They were air-mailed to India and stored for one month before use.

strain was used in the experiment. The results showed that for this particular strain the vitamins did not replace the action of the blood fractions, while an addition of vitamins to the blood fractions did not appear to hasten or enhance the development of colonies.

At a later date a small quantity of biotin was obtained through the kindness of Dr. I. F. Huddleson and the growth promoting effect of the four vitamins was tested against three different strains of *Brucella* in Maculloch's fluid, in salt agar with and without Maculloch's salts, in silica gels and in silica gels with Maculloch's salts added. A very insignificant deficient growth was noted only in salt agar and in silica gel both enriched with Maculloch's salts and the vitamins. As in previous experiments the *suis* strain germinated more readily than the *abortus,* while Str. 19 germinated doubtfully or not at all.

None the less, the absence of growth on the addition of the four vitamins to deficient media cannot with safety be compared with the growth resulting from the addition of blood fractions to defective or deficient media; for, with the blood, many nutrients, gross and otherwise, are added, which might well participate in the phenomenon. With the object of clarifying this point the four vitamins were added to defective liver agar which, apart from the presence of inhibitor, must contain many of the nutrients present in heated blood. The results showed that the presence of the vitamins added in double the amount recommended by Maculloch, did not cause a significant shift of the break point. Therefore, unless the additions were too small, or the vitamins had suffered during transport, it is difficult to identify any one of them with the inhibitor cancelling factors of blood.

In the first two sections of this paper an attempt has been made during the summer of 1944. During the winter, it was intended to devise a means of assaying the activity of the blood of various animals. This project had to be abandoned because all blood fractions, whether of rabbit or bovine origin, failed to give growth or gave such insignificant germination as to render comparison unreliable. In the spring of 1945 fresh attempts were made and an improvement of blood activity was noted. However, up to the time of writing it has not been possible further to examine these apparent variations.

## DISCUSSION

In the first two sections of this paper an attempt has been made to ascertain the nature of the break phenomenon and two separate functions have been noted, *viz.*, an inhibition of germination by a factor in the liver medium on the one hand, and a bacterial synthesis of a substance which overcomes this inhibition on the other. It remains to be seen, therefore, whether these two functions can of themselves account for the break phenomenon.

On general grounds it can be fairly safely postulated that the "anti-inhibitor" is first synthesised during the initial stages of cell

division and also that the cells of a given seeding will have a varying capacity to synthesise. It is possible, for instance, that actively dividing cells can synthesise faster than others. Now the amount of inhibitor present in a given series of defective cultures must be approximately equal in all plates and, in a series where the break occurs in plate 3, there is clearly enough inhibitor in plate 3 to block the growth of $10^7$ cells—possibly a little more. Since, as far as the eye can judge, germination in plate 2 is complete, there is apparently insufficient inhibitor in this plate to be lethal when divided throughout a $10^8$ population. It has already been said that, if inhibition alone was concerned in this phenomenon, then, in one or another plate of a close range series interpolated between plates 2 and 3, a population ought to occur, which is only some hundreds of thousands greater than that which the inhibitor can exactly neutralise and in this plate and in one or two preceding it growth would only be partial. It has also been noted that this sort of "tailing off" of growth does not occur as long as seedings are dense and inhibition is nearly maximal. What, in fact, does arise in these interpolated threshold plates is the development of a few atypical groups of colonies, which consist of a central well developed colony surrounded by minute satellites. It would appear that owing to small irregularities in the composition of the medium or to variations in the synthetic powers of indivdual cells, a cell here and there has managed to germinate belatedly and has then been able to synthesise sufficient anti-inhibitor to induce a few adjacent cells to develop later and thus give rise to incomplete colonies. Turning now to the plates of the interpolated series leading up to plate 2, it is clear that here the serial increase of population will be such that less and less inhibitor will be available per cell until a relation will occur which will permit several hundreds of the better synthesising cells to germinate. It follows from the experiments on satellitism that these cells will synthesise sufficient anti-inhibitor to enable many cells around them to germinate, which in their turn will satellise others and so on until the whole plate has germinated totally. On either side of a certain critical point, therefore, germination will be "all or nothing." It is evident that time is a very important factor in this reaction, for it has been seen that after five days' sojourn in defective cultures the cells die. It is clear, therefore, that synthesis of anti-inhibitor must take place early in the period of incubation in order that the majority of cells may be satellised before death supervenes. It is evident, also, that satellitism can operate most effectively where cells are very close together and it is perhaps for this reason that sharp total breaks occur mainly in densely seeded plates. It seems then that the functions which have been demonstrated experimentally can be fitted together to explain the phenomenon, whilst it is difficult to think of any other postulates that are equally satisfying.

A possible but attractive elaboration of the hypothesis, however,

would be to suppose that not only does anti-inhibitor cancel the inhibitor but that it is also a substance essential to germination, *i.e.*, a synthesised growth factor. It is a simple step to suppose that the reason why excess of inhibitor prevents germination is because it neutralises all this essential factor as fast as the seed cell synthesises it, a process which eventually causes death from exhaustion. The last concept is supported by the observation that the strains which satellitise, and presumably, therefore, synthesise, well are the very strains which are least inhibited by defective liver media. It may not be without significance that these strains are also the most virulent. It seems probable, for instance, that virulence is not unconnected with ability to survive and germinate, which in turn depends upon a capacity to synthesise anti-inhibitor or growth factor.

In this connexion another interesting point arises, namely, that if the reaction between anti-inhibitor and inhibitor is quantitative, it seems clear that *suis* strains can synthesise some 100,000 times more effectively than Str. 19, since in a normal defective preparation, Str. 19 generally breaks in plate 2 or 3 while S.1 breaks in plate 7 or 8. Conversely, if in two different defective preparations S.1 breaks in the one at plate 8 and in the other at plate 3, then the second preparation must contain inhibitor some 100,000 times more potent than the first. It follows that if potency is related to quantity then the substance in question must obviously be a trace-factor. That these relations are quantitative rather than qualitative seems likely from the regular pattern of the breaks when Str. 19, A.1, M.1, and S.1 are plated in the same defective preparation. If the foregoing hypothesis is correct then, whenever a true break is noted in cultures of a dilution series, bacterial inhibition and counter-synthesis can be deduced.

Perhaps the most interesting of the findings is the apparent seasonal fluctuation of the *Brucella* growth factors of blood. However, a very guarded view is taken of these results, and all that can be said at present is that several blood samples, taken at random from various animals during the summer, yielded germinal fractions so regularly that their presence was taken for granted. In the winter season, on the other hand, several random blood samples were wholly or nearly non-nutrient in basal media. If these observations are repeatable and not due to errors of technique, it must be inferred that the *Brucella* growth factors of blood fluctuate seasonally.

The occurrence of apparent breaks in the germination of genera other than *Brucella* is significant. If they are, indeed, true breaks, it is for future experiment to decide whether a single common inhibitor is responsible for the inhibition of several genera, or whether each genus has its own inhibitor. In this connexion reference may be made to the work of Webster (1925) and of Webster and Baudisch (1925) on "The Biology of *Bacterium lepisepticum*" in which a

break in the germination of *Pasteurella* dilution cultures attributed by Webster to a deficiency of the X factor, was noted. Webster, however, failed to question the remarkable fact that $10^4$ cells died whereas $10^9$ germinated, a phenomenon which cannot be explained on the basis of simple deficiency. It is evident that, in identical environments, the individual of a $10^4$ population is manifestly less deficient than that of a $10^9$ population and certainly less than that of a $10^9$ community. If the possibility that a growthfactor was carried over with the seed is set aside, in Webster's work also inhibition and counter-synthesis must be postulated. It must be accepted that the inhibitor belonged to the medium and it is virtually certain that it was derived from the beef. Clearly, if it had been attached to, or was a product of, the seed, the inhibitor would have been proportional to seed density and a break could not, in these circumstances, have occurred.

The most fascinating aspect of Webster's work is his demonstration of the connexion between the disappearance of the lag phase and the by-passing of inhibition by blood additions. It is possible that the lag phase represents a period of counter-synthesis.

The most perplexing part of the enquiry is undoubtedly the identity of the *Brucella* growthfactors of blood. The activity of the R.B.C. portion cannot fail to suggest cozymaze I and II. These substances, however, are relatively heatstable and cozymaze I, at least, is rapidly destroyed when the blood corpuscles are laked. Conversely, the *Brucella* growthfactor of R.B.C. appears to be sharply heatlabile and it can be stored in the refrigerator for weeks without losing potency. On the count of its heatstability the factor in plasma can hardly be identified with that of the R.B.C., while the latter cannot easily be connected with Koser's combination of vitamins.

## SUMMARY

In some attempts to count serially diluted *Brucella* in liver agar it was observed that the bacterial dilutions $10^{-1}$ and $10^{-2}$ (original suspension density = Brown's scale 5) germinated totally, whilst dilutions $10^{-3}$ and upwards failed to germinate at all.

The reasons for this abrupt cessation of orderly germination were sought. It was shown that during the gentle hydrolysis of liver, a non-dialysable *Brucella* inhibitor was produced, which was capable of preventing germination when added to otherwise germinal cultures. It was also shown that various *Brucella* strains responded variously in the presence of the inhibitor. The potency of the inhibitor for any given strain appeared to depend on the amount of heat used in hydrolysis and/or the condition of the liver itself. The ultimate action of the inhibitor was bactericidal.

It was also shown that germinating *Brucella* produced a factor which nullified the effect of this inhibitor. This "anti-inhibitor" appeared to be associable solely with live germinating cells and

not with dead cells, live non-germinating cells or with cell washings.

Two fractions of blood were discovered which could replace the anti-inhibitor in nullifying the action of the inhibitor. Conversely, certain vitamins known to be essential for the germination of certain strains of *Brucella* could not replace the function of anti-inhibitor.

An hypothesis based on the interaction of inhibitor and anti-inhibitor was evolved which can explain the abrupt cessation of growth in liver agar cultures.

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REFERENCES

Duckworth, J. (1935). *Biochem. J.*, **29,** 656.
Huddleson, I. F. (1939). *Brucellosis in Man and Animals.* New York: Commonwealth Fund.
Huddleson, I. F., Wood, E., and Cressman, E. (1945). *Science,* **101,** 358.
Koser, S. A., Breslove, B. B., and Dorfman, A. (1941). *J. infect. Dis.*, **69,** 114.
Levinthal, W. (1918). *Z. Hyg. InfektKr.*, **86,** 1.
Maculloch, N. B., and Dic, L. A. (1943). *Proc. Soc. exp. Biol. N.Y.* **32,** 310.
Mitchell, C. A., and Moore, T. (1941). *Canad. J. comp. Med.*, **5,** 107.
Webster, L. T. (1925). *J. exp. Med.*, **41,** 571.
Webster, L. T., and Baudisch, O. (1925). *Ibid.*, **42,** 473.
Wilson, G. S., Twigg, R. S., Wright, R. C., Hendry, C. B., Cowell, M. P. and Maier, I. (1935). *Spec. Rep. Ser. Med. Res. Council,* 206. London: H.M. Sta. Off.

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# THE ANTIDOTAL TREATMENT OF ARSENICAL POISONING

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## INTRODUCTION

The losses sustained annually by farmers in Africa from arsenical poisoning of their livestock are in the aggregate very considerable, for the general employment of arsenical dipping fluids to control tick infestation, the use of sodium arsenite as a food-bait during recurrent invasions of locusts, and the general irresponsibility of native employees into whose hands quantities of this poison may fall, all contribute to a high incidence of accidental poisoning. Some idea of the extent of these losses may be formed from a consideration of the numbers of specimens received at the Kabete Laboratories each year for examination for the presence of arsenic; in 1939*, 166 were examined, of which 45 per cent. were positive. During, and immediately after, locust control operations in any area, there is usually a sharp rise in the number of suspected and confirmed cases of arsenical poisoning, and long after such operations have ceased occasional cases continue to arise from the ingestion by grazing cattle of unconsumed and forgotten dumps of bait containing sodium arsenite, the drinking of rain-water from old drums that have contained bait, or the careless use of contaminated sacks. In such circumstances it may be assumed that many animals die from acute poisoning without symptoms being observed or suspicion aroused as to the true nature of the cause of death.

Opinion as to the value of the different antidotal and other treatments of cattle poisoned by arsenic varies, particularly as regards the efficacy of antidotes administered once symptoms are well established and the desirability or otherwise of giving repeated doses. This must perplex not only the farmer but the veterinarian called in to advise him, and thus it was evident that the subject would bear further investigation.

The classical antidote for poisoning by arsenic, *viz.*, arsenic trioxide (white arsenic) or its salts is moist ferric hydroxide, recently prepared by precipitation from a solution of ferric chloride. Winslow (1907), Wynter-Blythe (1920), Lander (1926) and Wooldridge (1934) all recommend this treatment, and as recently as 1939 Hoare states that this is the most effectual antidote. In recent years, however, doubt as to its efficacy has been expressed in several quarters. Sollman (1927) considers chemical antidotes to be of doubtful value and quotes experienced pharmacologists as finding that ferric

* This work was commenced and largely completed in 1939, but was then interrupted by the outbreak of hostilities.

hydroxide, while slightly reducing the percentage of fatalities, merely postpones death for a few hours in the majority of cases. Steyn (1939) states that he considers the administration of freshly prepared ferric hydroxide to be a waste of time and on the basis of experiments conducted at Ondestepoort advocates the use of sodium thiosulphate (hypo) orally and intravenously.

## EXPERIMENTAL

The arsenic used in this work was administered in the form of sodium arsenite (80 per cent. $As_2O_3$) as a drench in about half a pint of water. A satisfactory solution was obtained by heating.

The ferric hydroxide was prepared by diluting 100 c.c. of tincture of ferric chloride (B.P.) with 200 c.c. of water and adding sodium bicarbonate until effervescence ceased. The resulting suspension was immediately given as a drench. In all cases in which this antidote was employed three doses were given at intervals of about twenty minutes.

The sodium thiosulphate was given both by the mouth and by intravenous injection. When used as a drench the smallest dose given was 12 gm. to an animal weighing 190 lb. An additional 1 gm. was given for every 35 lb. increase in weight, so that the heaviest steer (613 lb.) received just over 24 gm. These quantities were dissolved in about half a pint of water. When given intravenously the smallest dose used was 2 gm. in 20 c.c. of water for the 190 lb. animal, and a third of a gram was added for every 35 lb. increase, so that the heaviest animal (613 lb.) received 5·6 gm. The amount of water in which the injection was dissolved was increased proportionately. While the quantities given were constant the periods between repeated doses varied.

Purgatives. Most authors advise the use of purgatives in arsenical poisoning, although there are some dissentients. In these experiments a half to a pint of raw linseed oil was given in each case one hour after the completion of the ferric hydroxide dosage and one hour after the final oral dose of sodium thiosulphate.

### *Estimation of a Standard Lethal Dose*

It was necessary to establish a standard toxic dose on which to base subsequent work on antidotes. The fatal single dose of white arsenic for farm animals as stated by different authors varies widely but in this connection it must be borne in mind that the toxicity of the agent depends chiefly on its solubility, and this is governed partly by the fineness of its state of division. Hoare (1939) writes: "4-8 drachms (16-32 gm.) are stated to produce poisonous effects in cattle," but it is not clear to what form of arsenic he is referring. Lander (1926) quotes Kauffmann as stating that the toxic dose of arsenious oxide in powder form for the ox is 225-700 grains (15-46 gm.). Wynter-Blythe, however, considers 10 grains (0·66 gm.) of solid arsenic (probably white arsenic) to be a dangerous dose for a cow. Theiler

(1912) found that up to 45 grains (3 gm.) of white arsenic given as the sparingly soluble powder had no ill-effect on a number of sheep weighing from 38 to 80 lb. Steyn and Bekker (1938) state that 2-4 pints of "three day dipping fluid" would be fatal to a full-grown beast and a quarter of this amount to a sheep (this represents about 1-2 gm. of arsenious oxide in solution for a beast and 0·25-0·5 gm. for a sheep). These authors also found the toxic dose for rabbits of various arsenical preparations, when calculated on the basis of their $As_2O_3$ content, to be 0·014 gm. $As_2O_3$ per Kg. bodyweight.

Apart from the actual amount of arsenic given, however, a further consideration is the length of time taken by an animal to die after the administration of a standard lethal dose. As acute arsenical poisoning only was to be investigated, a period of three days was selected as providing a reasonable opportunity for the administration of antidotal treatment, yet leaving no room for doubt that untreated animals would die. Accordingly, five healthy young steers were drenched with decreasing amounts of sodium arsenite. Table 1 presents the weights of the animals, the amounts of $As_2O_3$ given as sodium arsenite and the time taken to die.

TABLE I

| *No.* | *Weight, lb.* | *$As_2O_3$* | *Time of death after administration* |
|---|---|---|---|
| 4213 | 269 | 8·0 gm. | Within 3 hours |
| 4212 | 266 | 6·0 „ | „ 20 „ |
| 4229 | 298 | 4·0 „ | In 21 „ |
| 4231 | 290 | 2·4 „ | „ 36 „ |
| 4193 | 252 | 1·2 „ | „ 3½ days |

Thus it will be seen that the nearest approach to the required lethal dose was the case of 4193, which died 3½ days after a dose of 1·2 gm. of $As_2O_3$, or 0·59 gm. sodium arsenite per 100 lb. bodyweight. From this the standard lethal dose of sodium arsenite was fixed at 0·5 gm./100 lb., and subsequent work showed this amount to be satisfactory for the purpose.

### RESULTS OF ANTIDOTAL TREATMENT

Treatment was commenced at periods varying from five minutes to twenty-four hours after administration of the poison. The animals were housed in pens with stone floors and cement-faced walls; bedding was withheld and water given only sparingly. Results are summarised in Table 2.

*Group* 1. Treatment was commenced five minutes after the administration of the sodium arsenite. The three doses of ferric hydroxide were given to one animal by the mouth, while the other received sodium thiosulphate orally and intravenously. The sodium thiosulphate was repeated by both routes at the fifth hour and again intravenously only at the ninth hour. The control animal died in three days, whereas both treated steers survived, although they exhibited abdominal pain, inappetence, salivation and diarrhoea for

three or four days, after which recovery occurred. It is evident that neither antidote was able entirely to prevent absorption although they were administered more promptly than would be possible in almost any case of accidental poisoning.

*Group* 2. When commenced thirty minutes after the poison, the ferric hydroxide course of treatment did not prevent death although the animal lived 24 hours longer than the control. The animal which received the combined oral and intravenous sodium thiosulphate treatment after a similar delay and a second intravenous dose 20 hours later, showed the usual train of toxic symptoms for six days and became normal by the ninth day.

*Group* 3. In this group treatment was commenced after a delay of one hour. A further variation was that one steer was given the usual ferric hydroxide course followed by the intravenous injection of sodium thiosulphate at the 2nd, 7th and 23rd hours. Neither this animal nor the one which received ferric hydroxide alone was able to withstand the effects of the poison: eventually both died, although they lived three days longer than the control. The animal which received thiosulphate treatment alone, comprising an initial dose by the oral and intravenous routes followed by an intravenous dose at the 21st hour, recovered in nine day after showing the usual symptoms of poisoning.

*Group* 4. Dosing was commenced after 2½ hours. The steer which was given the three doses of ferric hydroxide was destroyed on humanitarian grounds after six days. The usual symptoms of acute arsenical poisoning were exhibited, but on the fourth day there appeared to be an improvement, and forcible feeding (milk and sugar as a drench) was adopted combined with a course of stimulating powders. Later a relapse occurred necessitating destruction. The animal receiving thiosulphate was given a second dose by the dual route at 6½ hours and an intravenous dose at 23½ hours. Recovery commenced on the fourth day and was complete by the seventh.

*Group* 5. In this group antidotal measures were commenced four hours after giving the poison. The animal given the ferric hydroxide course died in eighty hours. The steer which received thiosulphate at the 4th, 8th and 24th hours recovered after four days' illness.

*Group* 6. This experiment was similar to the preceding one except that antidotal treatment was withheld for twelve hours. The steer which received the three doses of ferric hydroxide died in four days, while the one which received multiple doses of thiosulphate recovered after eight days.

*Group* 7. When antidotal treatment was delayed for 18 hours neither ferric hydroxide nor multiple doses of sodium thisulphate was able to prevent death.

*Group* 8. This group differed from the previous one only in that treatment was withheld for 24 hours. Both treated animals died in 80 hours.

TABLE II

THE RELATIVE VALUE OF FERRIC HYDROXIDE (F.H.) AND SODIUM THIOSULPHATE (S.TH.) AS ANTIDOTES TO A SINGLE DOSE OF 0·5 GRAMME OF SODIUM ARSENITE PER 100 LB. BODY WEIGHT

| *No.* | *Weight, lb.* | *Antidote* | *Interval between antidote and sodium arsenite* | *Amount of antidote given* | *Remarks* |
|---|---|---|---|---|---|
| Group 1 | | | | | |
| 4566 | 386 | S.Th. | 5 minutes | 17·60 gm. orally 3·68 gm. I.V. | Recovered |
| | | | 5 hours | 17·60 gm. orally 3·68 gm. I.V. | |
| | | | 9 „ | 3·68 gm. „ | |
| 4562 | 400 | F.H. | 5 minutes | 3 doses at 20 min. intervals | Recovered |
| 4561 | 397 | Control | | No treatment | Died in 73 hours |
| Group 2 | | | | | |
| 6112 | 260 | S.Th. | 30 minutes | 14·00 gm. orally 2·66 gm. I.V. | Recovered |
| | | | 20½ hours | 2·66 gm. „ | |
| 3917 | 268 | F.H. | 30 minutes | 3 doses at 20 min. intervals | Died in 4 days |
| 6114 | 282 | Control | | No treatment | „ 72 hours |
| Group 3 | | | | | |
| 6852 | 532 | S.Th. & F.H. | 1 hour | 3 doses F.H. at 20 min. interval | Died in 6 days |
| | | | 2 hours | 4·93 gm. S.Th. I.V. | |
| | | | 7 „ | 4·93 gm. ditto | |
| | | | 23 „ | 4·93 gm. ditto | |
| 6407 | 476 | S.Th. | 1 hour | 21·80 gm. orally 4·93 gm. I.V. | Recovered |
| | | | 21 hours | 4·93 gm. I.V. | |
| 6849 | 578 | F.H. | 1 hour | 3 doses at 20 min. intervals | Died in 6 days |
| 6409 | 502 | Control | | No treatment | „ 74 hours |
| Group 4 | | | | | |
| 4387 | 301 | S.Th. | 2½ hours | 15·20 gm. orally 2·95 gm. I.V. | Recovered |
| | | | 6½ „ | 15·20 gm. orally 2·95 gm. I.V. | |
| | | | 23½ „ | 2·95 gm. „ | |
| 6846 | 451 | F.H. | 2½ „ | 3 doses at 20 min. intervals | Killed *in extremis* on 6th day |
| 6847 | 354 | Control | | No treatment | Died in 76 hours |
| Group 5 | | | | | |
| 3865 | 301 | S.Th. | 4 hours | 15·20 gm. orally 2·95 gm. I.V. | Recovered |
| | | | 8 „ | 15·20 gm. orally 2·95 gm. I.V. | |
| | | | 24 „ | 2·95 gm. „ | |
| 3820 | 402 | F.H. | 4 „ | 3 doses at 20 min. intervals | Died in 80 hours |
| 3870 | 426 | Control | | No treatment | „ 70 „ |

TABLE II—*continued*

| *No.* | *Weight, lb.* | *Antidote* | *Interval between antidote and sodium arsenite* | *Amount of antidote given* | *Remarks* |
|---|---|---|---|---|---|
| Group 6 | | | | | |
| 4177 | 613 | S.Th. | 12 hours | 24·10 gm. orally<br>5·63 gm. I.V. | Recovered |
| | | | 16 „ | 24·10 gm. orally<br>5·63 gm. I.V. | |
| | | | 22 „ | 24·10 gm. orally<br>5·63 gm. I.V. | |
| | | | 36 „ | 5·63 gm. „ | |
| 6845 | 460 | F.H. | 12 „ | 3 doses at 20 min. intervals | Died in 4 days |
| 6848 | 474 | Control | | No treatment | „ 68 hours |
| Group 7 | | | | | |
| 5896 | 190 | S.Th. | 18 hours | 12·00 gm. orally<br>2·00 gm. I.V. | Died in about 4 days |
| | | | 22 „ | 12·00 gm. orally<br>2·00 gm. I.V. | |
| | | | 26 „ | 12·00 gm. orally<br>2·00 gm. I.V. | |
| | | | 42 „ | 2·00 gm. „ | |
| 5776 | 181 | F.H. | 18 „ | 3 doses at 20 min. intervals | Died in about 4 days |
| 4646 | 241 | Control | | No treatment | Died in about 4 days |
| Group 8 | | | | | |
| 5821 | 272 | S.Th. | 24 hours | 14·30 gm. orally<br>2·70 gm. I.V. | Died in 80 hours |
| | | | 28 „ | 14·30 gm. orally<br>2·70 gm. I.V. | |
| | | | 32 „ | 14·30 gm. orally<br>2·70 gm. I.V. | |
| | | | 48 „ | 2·70 gm. I.V. | |
| 6575 | 211 | F.H. | 24 „ | 3 doses at 20 min. interva)s | Died in 80 hours |
| 5522 | 231 | Control | | No treatment | Died in 68 hours |

### *The Effect of Immediate Antidotal Treatment on Animals Ingesting Five Times the Standard Lethal Dose*

In order to ascertain the possible value of antidotes in animals ingesting several times the standard lethal dose, two steers were given five times the fatal dose of sodium arsenite used in these experiments and antidotal treatment commenced as quickly as possible. Thus five minutes later one was given ferric hydroxide by the mouth and the other sodium thiosulphate orally and intravenously. In both cases oral treatment was twice repeated at half-hour intervals, and, in addition, the animal receiving thiosulphate was given an intravenous injection six hours after being dosed with the arsenite. Both died from acute poisoning within 24 hours.

## DISCUSSION

It is evident that the amount of a single dose of arsenic in the

form of sodium arsenite in solution which is sufficient to kill cattle, is less than that stated by some authors (Hoare, 1939; Lander, 1926), although the large amounts they quote probably refer to $As_2O_3$ in its comparatively insoluble form. In the present experiments 0·4 gm. of $As_2O_3$ per 100 lb. bodyweight killed healthy young animals in about three days. If the average weight of adult native cattle is put at 600 lb. this dose represents 2·4 gm. It is probable that even smaller amounts would prove lethal, although the time taken to die would be longer.

These findings correspond closely with those of Steyn and Bekker (1938 who found that ± 1 to ± 2 gm. given as "three day dip" proved fatal to a full-grown beast. Wynter-Blythe (1920), on the other hand, considers that 10 grains (0·66 gm.) of solid arsenic is a dangerous dose for a cow. If by solid arsenic is meant powdered white arsenic, this figure would appear to be on the low side, for on one occasion a dose of 0·25 gm./100 lb. of sodium arsenite was given to a young beast without any effects more serious than a few days' illness. This represents 1·5 gm. for a 600 lb. cow.

It is not possible to make exact comparisons between the two forms of antidotal treatment because under the conditions of the experiments they were not administered with equal frequency. It will be seen, however, that when ferric hydroxide was used in the form of three oral doses at intervals of 20 minutes and followed by a purgative one hour later, death was prevented only when treatment was commenced a few minutes after the administration of the poison. Sodium thiosulphate, on the other hand, when given by the mouth and by intravenous injection and repeated on from one to three occasions during the following 36 hours, was effective in preventing death even when treatment was delayed up to 12 hours after giving the poison.

Since the findings previously quoted are based on the ingestion of a quantity of sodium arsenite sufficient to cause death in three days, and whereas an animal at pasture may have consumed an amount sufficient to cause subacute poisoning only, it is suggested that under field conditions thiosulphate treatment should not be abandoned even when there has been a delay of longer than 12 hours. It is also possible that persistent intravenous injections of thiosulphate, by combating the poison already absorbed, might have the effect of saving an animal's life when it is considered too late to adopt drenching. Nevertheless it is clear that neither antidote is likely to be effective when several times the standard lethal dose has been consumed.

In the experiments reported above symptoms did not appear for six to nine hours after ingestion of the standard lethal dose and even 24 hours later they were not striking. When five times the standard dose was given and death occurred in 20 hours, the onset of symptoms was delayed for three hours. It is clear, therefore, that the application of antidotal treatment in acute cases of poison-

ing is unlikely to be successful if withheld until clinical symptoms are well advanced.

During the experiments a moderate quantity of water was allowed to the animals because of the copious diarrhoea which occurred. The clinical picture and the post-mortem findings in all cases were in close agreement with the observations of other workers.


## SUMMARY

Arsenic administered as a drench in the form of a solution of sodium arsenite in a dosage of 0·4 gm. $As_2O_3$ per 100 lb .bodyweight caused the death of healthy young steers after about 3½ days, and this dose was employed in the assessment of the value of antidotal treatment.

Ferric hydroxide freshly prepared by the addition of sodium bicarbonate to a solution of ferric chloride and given as a course of three doses at twenty-minute intervals by drenching followed one hour later by a purgative, was effective in preventing death only when treatment was commenced five minutes after the administration of the poison.

Sodium thiosulphate, given to steers varying from 190 to 613 lb. in weight, in doses of 12-24 gm. by the mouth, and in quantities of 2·0-5·6 gm. by intravenous injection, the doses being repeated up to four times at varying intervals, was effective in preventing death when treatment was commenced at periods varying from a few minutes to twelve hours after the administration of the sodium arsenite.

Similar thiosulphate treatment failed when commenced 18 hours after ingestion of the poison, as also did both forms of antidotal treatment when applied almost immediately after the administration of five times the standard lethal dose.

It is suggested that under field conditions where the quantity of arsenic consumed may be smaller than the standard dose used in these experiments, sodium thisulphate may be successful even if administration is delayed beyond the twelfth hour.

The experiments indicate that in acute arsenical poisoning a hopeful prognosis cannot be given if treatment is delayed until the onset of marked clinical symptoms.



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## REFERENCES


Hoare, W. (1939). *Hoare's Veterinary Therapeutics,* 5th edit. London: Baillière, Tindall and Cox.

Lander, G. D. (1926). *Veterinary Toxicology,* 2nd edit. London: Baillière, Tindall and Cox.

Sollmann, T. (1927). *A Manual of Pharmacology.* London: Saunders.
Steyn, D. G. (1939). *Farming in South Africa,* **14,** 105.
Steyn, D. G. and Bekker, P.M. (1938). *Ondestepoort, J.,* **11,** 247
Theiler, A. (1912). *Agriv. J. Un. S. Afr.,* **3,** 321.
Winslow, K. (1907). *Veterinary Materia Medica and Therapeutics,* 5th edit. London: Baillière, Tindall and Cox.
Wooldridge, G. H. (1934). *Encyclopaedia of Veterinary Medicine and Obstetrics;* 2nd edit. Vol. I. Oxford Univ. Press, London: Humphrey Milford.
Wynter-Blythe, A. (1920). *Poisons, their effects and detection;* 5th edit.; London: Griffin.

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# PREVALENCE OF BOVINE MASTITIS IN EGYPTIAN DAIRY CATTLE

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## INTRODUCTION

It is generally agreed that mastitis is one of the most widespread diseases of cattle, and is of great economic importance to the farmer and the milk industry. In the acute form the cow may lose one or more quarters or become quite valueless as a secreting animal, whilst in the widespread chronic form the milk from infected udders is not only considerably reduced in amount but may also be unfit for consumption. The changes produced in the quality of milk may not render it grossly abnormal, but may alter its palatability and marketability and also make it unsuitable for various manufacturing purposes, particularly butter-making.

Some idea of the losses involved may be obtained by considering the figures given by certain research workers. Pröscholdt (1928), examined 1,126 herds in North Germany and found that 50 per cent. were infected by *Strept. mastitidis*. Klimmer (1929) estimated the annual loss in Germany as equivalent to ten million pounds sterling which, as there were about ten million dairy cows in Germany at that time, meant a loss of £1 sterling per cow per annum. German and Austrian authorities before the war assessed their losses from streptococcal mastitis at one-third the value of their annual milk production. Shaw and Beam (1935) found that the decrease in the yield of infected as compared with non-infected quarters, amounted to 22 per cent. in milk and 24 per cent. in butter fat. Rosell reported that 57,190 cows with mastitis are eliminated annually in New York State, and that the combined losses of secreting cows and milk totalled approximately 49 million dollars a year in that State alone. It has been estimated that the losses from mastitis there exceeded those caused by either tuberculosis or Bang's disease. Stableforth (1939) produced figures to show that the loss per infected cow varied from £2 to £5 sterling, depending on its normal milk yield, and that, taking the number of dairy cows in Great Britain as 2,700,000 and the percentage infected as 20, the total loss from lowered milk yield alone would be from £1,080,000 to £2,700,000 sterling per annum.

In addition, certain streptococcal infections of the cow's udder have long been suspected as the source of infection in cases of scarlet fever and septic sore throat in man. Capps and Miller (1852), in their investigation of the Chicago outbreak of septic sore throat, found that 4·6 per cent. of the cows supplying milk to the dairy plant under suspicion had mastitis. Wahby (1932), accumulating data on milk epidemics, reported that in the U.S.A. there had occurred, up to 1929, 49 epidemics of sore throat involving 22,545 cases and 87 epidemics of scarlet fever involving 8,358 cases; and in England 21 sore throat epidemics involving 2,039 person and 88 epidemics of scarlet fever affecting 7,650 persons. Some of these streptococcal infections undoubtedly originated from infected quarters, and these figures clearly show

that mastitis may be highly dangerous to the public health when caused by streptococci of human origin.

On account of the sanitary and economic importance of the disease the work described in this paper was undertaken in order to determine the extent to which it occurs among Egyptian milk-producing cattle.

## METHODS

Five hundred individual milk samples from 450 buffaloes and 50 cows were collected from dairy farms and stables in different sections of Cairo and its suburbs. The samples consisting of about 100 c.c., 25 c.c. from each quarter, were drawn as aseptically as possible. The physical condition of the milk was noted and recorded and the cow's udder clinically examined.

The samples were submitted to (1) indirect or subsidiary tests as specified below and (2) bacteriological examination, including the Hotis test.

### *Indirect tests*

As these could not usually be carried out immediately, the samples were stored in the ice-chest for periods not exceeding 12 hours after collection.

*Hydrogen-ion concentration* was tested by means of bromthymol blue solution using the technique recommended by Case (1937) for the determination of the pH of mastitis milk.

*Determination of chlorides.*—Two tests were used, (i) Volhard-Harvey's method recommended by Hadyen (1932), and (ii) Drost's method as modified by Bischoff (1929).

*Leucocyte count.*—For determination of the number of leucocytes in milk samples, Skar's (1926) method for bacterial counts was used.

### *Bacteriological examination*

The samples were examined in most cases within two hours of collection, the methods used being the Hotis test and cultivation of centrifuged deposits. After 24 hours' incubation suspicious or positive Hotis tubes were examined microscopically and plated out on nutrient agar. The plates were incubated for 48 hours and examined for the presence of streptococci which were isolated and identified by their reactions in lactose, glucose, saccharose, maltose, mannite, raffinose, inulin and salicin, and by their effects on blood agar, nutrient gelatin, litmus milk, methylene blue milk and sodium hippurate. Centrifuged sediments from 10 c.c. quantities of milk were examined microscopically and a loopful from each inoculated into broth was incubated for 48 hours. Films were then made and the culture plated out on nutrient agar. The plates were examined after 48 hours' incubation for the presence of colonies of streptococci which in turn were identified as described above.

*Staphylococcus aureus* (haemolytic type) was detected by the

inoculation of agar plates with centrifuged sediments; they were incubated for 48 hours and examined for typical orange-yellow colonies which were tested for haemolysis on blood agar. The haemolytic strains were further tested in sugar media, such as glucose, lactose, saccharose, maltose and mannite, and in litmus milk and gelatin. Special attention was also directed to the detection of *Corynebacterium pyogenes*. For this purpose blood agar plates were inoculated with a loopful of the culture of the sediment in broth and examined after 48 hours' incubation, but the organism was not recovered from any of the samples.

## RESULTS

### *Clinical inspection*

Examination of the udder proved in most cases to be an unreliable means of diagnosis. Owing to the marked variation in size and consistence of the quarters of the animals examined, it was often difficult to form an opinion as to the condition of the gland and in the absence of frank inflammatory signs, to decide whether it was diseased or healthy. Definite clinical symptoms were recognisable in only 32 (23 streptococcal due to *Str. agalactiae,* eight staphylococcal and one apparently traumatic) out of 121 cases of mastitis, *i.e.,* 26·4 per cent. The remainder of the cases were diagnosed as the result of bacteriological examination.

### *Indirect methods*

The testing of the milk by indirect methods was also unreliable for the diagnosis of chronic mastitis. Table 1 shows the results of applying these tests in the proved mastitis cases, and it will be seen, for example, that the chloride quantitative test (Drost's) gave a positive result (chloride $> 0{\cdot}14$ per cent.) in 18 of 121 mastitis cases, *i.e.,* 14·8 per cent. (identical figures were obtained by the use of Volhard-Harvey's method.) The leucocyte counts exceeded one million cells per c.c. of milk in 64 of the cases (50·9 per cent) and the reaction test gave positive results (pH 7·0 or above) in three samples only (2·4 per cent.). On the other hand, the leucocyte count exceeded one million cells per c.c. in 112 samples from normal udders. This was probably due to mechanical irritation or caused by rough milking technique.

The inaccuracy of the indirect tests experienced in this work may have been partially due to the fact that mixed milk obtained from the four quarters of each udder was used. In such composite samples the properties of the changed milk from diseased quarters would be obscured by milk derived from healthy quarters, and this would be additional to the inaccuracies inherent in these tests which have been pointed out by numerous authors (Stableforth, Edwards and Minett, 1935; Blood and Rowlands, 1936; Show, Hanson and Nutting, 1937; Klein, Blechner and Scheidy, 1938).

TABLE I
SHOWING THE RESULTS OF THE INDIRECT TESTS IN CASES OF MASTITIS

| *Serial No. of positive samples* | *Leucocyte count per c.c.* | *Chloride percentage* | *Reaction* | *Serial No. of positive samples* | *Leucocyte count per c.c.* | *Chloride percentage* | *Reaction* |
|---|---|---|---|---|---|---|---|
| 001 | 945,279 | 0·1134 | 6·5 | 128 | *1,260,372* | 0·1134 | 6·6 |
| 003 | *2,331,688* | 0·1206 | 6·6 | 133 | *1,386,409* | 0·1063 | 6·3 |
| 029 | 945,279 | 0·0638 | 6·5 | 136 | *1,008,297* | 0·0602 | 6·3 |
| 034 | *1,071,316* | 0·0709 | 6·6 | 137 | 945,279 | 0·0602 | 6·5 |
| 040 | *2,205,651* | 0·1277 | 6·5 | 144 | *1,260,372* | 0·0533 | 6·5 |
| 042 | *2,454,725* | *0·1602* | 6·5 | 149 | *882,262* | 0·0602 | 6·5 |
| 049 | *819,236* | 0·0886 | 6·5 | 150 | *1,134,334* | 0·0851 | 6·7 |
| 054 | *630,186* | 0·0638 | 6·5 | 153 | *1,008,297* | 0·0709 | 6·3 |
| 055 | *2,451,725* | 0·1063 | 6·5 | 154 | *1,008,297* | 0·0815 | 6·5 |
| 056 | 504,148 | 0·0815 | 6·5 | 155 | *1,260,372* | 0·0992 | 6·5 |
| 060 | *1,134,334* | 0·0993 | 6·5 | 162 | 819,236 | 0·0709 | 6·5 |
| 066 | *1,764,520* | 0·1063 | 6·5 | 165 | *1,134,334* | 0·0709 | 6·5 |
| 068 | *7,814,306* | *0·1560* | *7·0* | 169 | 882,262 | 0·0815 | 6·3 |
| 069 | *1,575,465* | 0·1063 | 6·7 | 171 | *1,071,316* | 0·0709 | 6·5 |
| 072 | *1,134,334* | 0·0886 | 6·5 | 175 | *1,386,409* | 0·0851 | 6·5 |
| 086 | *1,008,297* | 0·0992 | 6·5 | 176 | *1,008,297* | 0·0708 | 6·7 |
| 087 | 945,279 | 0·1134 | 6·4 | 180 | *1,008,297* | 0·0708 | 6·3 |
| 088 | *1,260,372* | 0·1276 | 6·5 | 184 | 630,186 | 0·0709 | 6·3 |
| 089 | 504,148 | 0·0533 | 6·5 | 190 | *1,008,297* | 0·0533 | 6·7 |
| 090 | *1,134,334* | 0·0602 | 6·3 | 192 | *1,008,297* | 0·0709 | 6·7 |
| 091 | *1,134,334* | 0·0602 | 6·3 | 196 | *1,008,297* | 0·0815 | 6·3 |
| 101 | *1,071,316* | 0·1134 | 6·3 | 207 | 945,279 | 0·0851 | 6·3 |
| 105 | *1,260,372* | 0·0992 | 6·5 | 213 | *1,701,502* | 0·0815 | 6·5 |
| 106 | *1,071,316* | 0·0992 | 6·4 | 214 | *1,701,502* | 0·0851 | 6·3 |
| 111 | 945,279 | 0·0886 | 6·3 | 215 | *1,701,502* | 0·0851 | 6·3 |
| 117 | *3,933,180* | *0·1666* | 6·4 | 216 | *1,134,334* | 0·1028 | 6·5 |
| 118 | 315,093 | 0·0533 | 6·5 | 220 | 630,186 | 0·0815 | 6·5 |
| 119 | *1,134,334* | 0·1241 | 6·5 | 223 | 945,279 | 0·0709 | 6·3 |
| 127 | *1,386,409* | 0·0992 | 6·5 | 226 | *1,449,427* | 0·0815 | 6·3 |
| 230 | *1,260,372* | *0·1483* | 6·5 | 309 | 315,093 | 0·1028 | 6·3 |
| 232 | 945,279 | 0·0927 | 6·3 | 310 | 882,262 | 0·1028 | 6·5 |
| 238 | 630,186 | 0·1028 | 6·3 | 322 | 756,232 | 0·0921 | 6·5 |
| 245 | *1,827,539* | 0·1028 | 6·5 | 339 | *1,197,353* | 0·0815 | 6·3 |
| 246 | *1,575,465* | 0·0709 | 6·5 | 340 | *1,269,372* | 0·0957 | 6·3 |
| 254 | 567,167 | *0·1483* | 6·5 | 344 | *1,638,483* | 0·0602 | 6·5 |
| 255 | 945,279 | 0·0602 | 6·3 | 352 | 756,223 | 0·0957 | 6·5 |
| 256 | *3,907,153* | *0·1524* | 6·7 | 353 | *1,890,558* | 0·0815 | 6·3 |
| 257 | *2,331,611* | 0·0602 | 6·5 | 359 | 765,223 | 0·0815 | 6·3 |
| 260 | 693,204 | 0·0502 | 6·5 | 363 | 252,074 | 0·0709 | 6·5 |
| 263 | 315,093 | 0·0533 | 6·3 | 365 | 441,130 | 0·0638 | 6·5 |
| 264 | *1,071,316* | *0·1483* | 6·3 | 373 | 467,167 | 0·0886 | 6·5 |
| 265 | 882,262 | 0·0533 | 6·3 | 375 | 576,167 | 0·0709 | 6·5 |
| 266 | 441,130 | 0·0609 | 6·3 | 382 | 756,223 | 0·0815 | 6·5 |
| 268 | 630,186 | *0·1524* | 6·5 | 384 | *1,575,464* | 0·1063 | 6·5 |
| 271 | 441,302 | 0·0609 | 6·5 | 400 | 504,148 | 0·0992 | 6·5 |
| 278 | *1,008,297* | 0·0815 | 6·3 | 407 | 315,093 | 0·0709 | 6·5 |
| 281 | *1,260,372* | *0·1483* | 6·5 | 423 | 252,074 | 0·0709 | 6·3 |
| 283 | *1,764,520* | *0·1524* | 6·5 | 426 | *2,772,818* | *0·1483* | 6·5 |
| 284 | 504,148 | *0·1524* | 6·3 | 428 | 315,093 | 0·0638 | 6·5 |
| 290 | *1,512,448* | *0·1483* | 6·5 | 430 | 252,074 | 0·0602 | 6·5 |
| 291 | *1,134,334* | *0·1483* | 6·3 | 432 | 252,074 | 0·0638 | 6·3 |
| 292 | *2,394,706* | 0·0921 | 6·7 | 433 | 630,186 | 0·0709 | 6·5 |
| 293 | *1,512,448* | 0·1028 | 6·5 | 435 | 567,167 | 0·0553 | 6·3 |
| 294 | 630,186 | 0·0602 | 6·6 | 439 | 378,111 | 0·0638 | 6·5 |
| 295 | *2,079,614* | *0·1453* | *7·2* | 448 | 378,111 | 0·0602 | 6·3 |
| 299 | 756,223 | 0·0780 | 6·5 | 454 | 378,111 | 0·0815 | 6·5 |
| 300 | 630,186 | 0·0780 | 6·3 | 455 | 441,130 | 0·0709 | 6·5 |
| 302 | *1,260,372* | *0·1602* | 6·5 | 474 | *2,394,756* | *0·1702* | *7·4* |
| 303 | 630,186 | 0·1063 | 6·3 | 480 | 630,186 | 0·0815 | 6·3 |
| 304 | 252,074 | 0·0815 | 6·6 | 493 | 882,262 | 0·0709 | 6·3 |
| 306 | *1,260,372* | *0·1524* | 6·5 | | | | |

*N.B.*—No. 42 is traumatic mastitis. Figures in italics are above the normal limits

*Bacteriological examination*

Cultural methods revealed that 120 cases were caused by bacteria, and one was apparently traumatic in origin (No. 42 in which no specific organism could be isolated). There were 109 streptococcal mastitis cases, of which 54 were due to *Str. agalactiae*, 23 to *Str. dysgalactiae*, 30 to *Str. uberis*, and 2 to *Str. epidemicus*. In addition, 11 cases of mastitis caused by *Staph. aureus* (haemolytic type) were detected. Table 2 shows the number of strains of pathogenic and non-pathogenic streptococci and *Staph. aureus* isolated from the series of samples by bacteriological methods.

As the bacteriological examination consisted of two main tests—the Hotis test and cultivation of milk sediment—their comparative value as revealed in this investigation may be briefly considered.

TABLE

ISOLATION OF STREPTOCOCCI AND

| *Pathogenic Streptococci* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Streptococcus agalactiae (*Haemolytic type*) | | | Streptococcus agalactiae (*Non-haemolytic type*) | | | Streptococcus dysgalactiae | | | Streptococcus uberis | | |
| *Hotis test alone* | *Centrifugal sediment alone* | *Hotis and centrifugal sediment* | *Hotis test alone* | *Centrifugal sediment alone* | *Hotis and centrifugal sediment* | *Hotis test alone* | *Centrifugal sediment alone* | *Hotis and centrifugal sediment* | *Hotis test alone* | *Centrifugal sediment alone* | *Hotis and centrifugal sediment* |
| 106 | 169 | 49 | 56 | 29 | 1 | 111 | 72 | 60 | 144 | 175 | 54 |
| 215 | 190 | 69 | 127 | 353 | 34 | 117 | | 101 | 149 | 180 | 153 |
| | 192 | 86 | 133 | | 40 | 136 | | 154 | 213 | 184 | 293 |
| | 216 | 87 | 137 | | 66 | 230 | | 278 | 246 | 196 | 423 |
| | 407 | 88 | 162 | | 89 | 232 | | 281 | 299 | 207 | 439 |
| | | 220 | 214 | | 90 | 238 | | 283 | 310 | 448 | |
| | | 245 | 226 | | 91 | 254 | | 293 | 322 | 454 | |
| | | | 257 | | 105 | 260 | | | 340 | 493 | |
| | | | 263 | | 119 | 268 | | | 344 | | |
| | | | 265 | | 128 | 284 | | | 373 | | |
| | | | 271 | | 150 | 290 | | | 375 | | |
| | | | 294 | | 165 | 291 | | | 428 | | |
| | | | 304 | | 232 | 302 | | | 340 | | |
| | | | 339 | | 255 | 306 | | | 432 | | |
| | | | 352 | | 266 | 359 | | | 433 | | |
| | | | 363 | | 278 | | | | 435 | | |
| | | | 365 | | 300 | | | | 455 | | |
| | | | | | 309 | | | | | | |
| | | | | | 384 | | | | | | |
| | | | | | 400 | | | | | | |
| | | | | | 480 | | | | | | |
| 2 | 5 | 7 | 17 | 2 | 21 | 15 | 1 | 7 | 17 | 8 | 5 |
| | 14 | | | 40 | | | 23 | | | 30 | |
| | | | | | | 23 clinically recognised cases | | | | | |

*Hotis test.*—This was first developed by Hotis and Miller (1936), who claimed that it was specific for the presence of *Str. agalactiae.* This investigation indicates, however, that samples which show change of colour are not necessarily derived from diseased udders Of 296 samples which showed positive change of colour, 172 contained no streptococci but only other cocci, sarcina, and lactobacilli. Of the 124 remaining samples, 92 contained pathogenic streptococci, namely, 9 *Str. agalactiae* (haemolytic), 38 *Str. agalactiae* (non-haemolytic), 22 *Str. dysgalactiae,* 22 *Str. uberis* and 1 *Str. epidemicus.* Of the rest of the samples, 31 contained non-pathogenic streptococci, such as *Str. bovis, Str. lactis* and *Str. liquefaciens* (table 2). Thus the Hotis test did not by itself give a final and definite result, and should therefore always be coupled with other

II

*Staphylococcus aureus* FROM MILK SAMPLES

| Streptococcus epidemicus | | | *Other Streptococci* | | | | | | | | | Staphylococcus aureus (*haemolytic*) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Streptococcus bovis | | | Streptococcus lactis | | | Streptococcus liquefaciens | | | |
| *Hotis test alone* | *Centrifugal sediment alone* | *Hotis and centrifugal sediment* | *Hotis test alone* | *Centrifugal sediment alone* | *Hotis and centrifugal sediment* | *Hotis test alone* | *Centrifugal sediment alone* | *Hotis and centrifugal sediment* | *Hotis test alone* | *Centrifugal sediment alone* | *Hotis and centrifugal sediment* | |
| 171 | 176 | — | 85 | 83 | 64 | 59 | 146 | 63 | 116 | 405 | 393 | 3 |
| | | | 228 | 173 | 288 | 129 | | 140 | 139 | | | 55 |
| | | | 233 | 174 | 437 | 157 | | | 141 | | | 68 |
| | | | 298 | | 438 | 159 | | | 296 | | | 118 |
| | | | 324 | | | 301 | | | 307 | | | 223 |
| | | | 398 | | | 305 | | | 341 | | | 256 |
| | | | 414 | | | 315 | | | 354 | | | 292 |
| | | | 452 | | | | | | 377 | | | 295 |
| | | | | | | | | | 436 | | | 303 |
| | | | | | | | | | | | | 426 |
| | | | | | | | | | | | | 474 |
| 1 | 1 | — | 8 | 3 | 4 | 7 | 1 | 2 | 9 | 1 | 1 | 11 |
| | 2 | | | 15 | | | 10 | | | 11 | | 11 |
| | | | | | | | | | | | | 8 were clinically recognisable |

bacteriological tests. On the other hand, it can be looked upon as a valuable preliminary test for the detection of streptococcal mastitis. Of 109 cases actually diagnosed, 92 were detected by this test, giving a percentage of 84·4.

*Cultivation of milk sediment.*—This method revealed the presence of pathogenic streptococci in 57 of the 109 samples which gives a percentage accuracy in the diagnosis of streptococcal mastitis of 52·3. The 57 strains of pathogenic streptococci comprised 12 *Str. agalactiae, (haemolytic)*, 23 *Str. agalactiae* (non-haemolytic), 8 *Str. dysgalactiae,* 13 *Str. uberis,* and 1 *Str. epidemicus* (Table 2). Of the 57 samples from which these strains were obtained 17 showed no change of colour in the Hotis test. The two tests were in agreement in only 40 samples. It is therefore recommended that both the Hotis test and the cultivation of milk sediment should always be used in the routine examination of milk for streptococcal mastitis.

Of the 500 dairy animals examined, 109 (21·8 per cent. showed pathogenic streptococci in their milk. Of the 450 dairy buffaloes 98 21·7 per cent.), and of the 50 cows 11 (22 per cent.), were affected; thus the incidence was about the same in the two species. The two strains of *Str. epidemicus* were isolated from buffaloes and the staphylococcal infections also occurred exclusively in this species.

## CONCLUSIONS

Individual milk samples obtained from 500 dairy animals (450 buffaloes and 50 cows) in Cairo and its suburbs have been examined for bovine mastitis.

A total of 121 cases was detected, a percentage incidence of 24·2. Of this number 109 (21·8 per cent.) were due to streptococci, *viz.,* 54 (10·8 per cent.) to *Str. agalactiae,* 24 (4·6 per cent.) to *Str. dysgalactiae,* 30 (6 per cent.) to *Str. uberis,* and 2 (0·4 per cent.) to *Str. epidemicus.* In addition 11 cases (2·2 per cent.) were due to *Staph. aureus* and 1 apparently to trauma.

The incidence of streptococcal mastitis was about the same in both buffaloes and cows.

Only 32 (26·4 per cent.) of the 121 cases were recognised clinically, of which 23 were due to *Str. dysgalactiae,* 8 to *Staph. aureus* and the remaining one was apparently traumatic.

The indirect tests gave unreliable results. The dependability percentage in respect of the positive samples was 50·9 for the leucocyte count, 14·8 for the chloride test and 2·4 for the reaction test.

The Hotis test can be regarded as a valuable preliminary means of detecting bovine mastitis. It revealed the presence of the disease in 92 out of 109 streptococcal cases (84·4 per cent.).

Cultivation of the milk sediments was less successful and detected 57 cases only (52·3 per cent.). Of these 17 were not detected by the Hotis test; the tests were in agreement in 40 cases only.

For the routine examination of individual milk samples for bovine

mastitis both tests should be employed in order to obtain the best results.

REFERENCES

Bischoff, O. (1929). *Dtsch. tierärztl Wschr.*, **37**, 147.
Blood and Rowlands. (1936). *Vet. Bull.*, **6**, 569.
Capps and Miller. (1852). *J. Amer. med. Ass.*, **58**, 1848.
Case, C. H. (1937). *ibid.*, **90**, 137.
Hadyen, S. E. (1932). *Cornell Vet.*, **22**, 277.
Hotis and Miller. (1936). *U.S. Dept. of Agric. Circ. No.* 400, p. 6.
Klein, Blechner and Scheidy. (1938). *Univ. Pa. Vet. Ext. Quart.*, **39**, 20.
Klimmer, M. (1929). *Tierärztliche. Milchkontrolle*, p. 25. Berlin: R. Schoetz.
Pröscholdt, O. (1928). *Arch. wiss. prakt. Tierheilk.*, **58**, 485.
Shaw and Beam. (1935). *J. dairy Sci.*, **18**, 353.
Show, Hanson and Nutting. (1937). *J. Amer. vet. med. Ass.*, **90**, 137.
Skar, O. (1926). *Ernst Grundriss der Milchhygiene.*, Stuttgart, p. 370.
Stableforth, A. W., Edwards, S. J., and Minett, F. C. (1935). *J. comp. Path.*, **48**, 300.
Stableforth, A. W. (1939). *Vet. Rec.*, **51**, 469.
Wahby, A.M. (1932). *J. Egypt. med. Ass.*, No. 12, 796.

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# OBSERVATIONS ON BOVINE MALIGNANT CATARRH IN PALESTINE

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## INTRODUCTION

In 1943, following clinical and laboratory examinations that left no doubt as to the identity of the disease, bovine malignant catarrh was added to the disease listed in the Animal Diseases Ordinance as notifiable, and an opportunity arose to study certain aspects of the disease as noted in cases reported by Government Veterinary Officers to the Government Veterinary Laboratory, Tel-Aviv.

The observations made are described under two headings:— (1) transmission of the disease to laboratory animals, and (2) histological findings in cases of the disease in cattle.

## MATERIAL AND METHODS

The material available for study consisted of eleven fatal and one non-fatal cases of the typical "head-and-eye" form of bovine malignant catarrh.

Bacteriological and histological examinations and filtration of material for small animal inoculations were carried out by standard methods.

## TRANSMISSION OF THE DISEASE TO LABORATORY ANIMALS

Workers in different countries have had varying results in their efforts to infect laboratory animals with the virus of malignant catarrh. Mettam (1923) failed to produce the disease in laboratory animals. According to Daubney and Hudson (1936), Dobberstein and Hemmert-Halswick (1928) and Stolnikoff (1933) failed to infect rabbits, while Götze and Lies (1929) could not transmit the disease to rabbits, mice, rats, or guinea-pigs. Bisbocci (1934) tried unsuccessfully to infect rabbits with emulsions of brain, aqueous humour and blood. Miessner and Schoop (1934) could not infect rabbits and guinea-pigs, and Aleska (1935) had inconclusive results with his experiments on rabbits, finally concluding that they were not susceptible. According to Magnusson (1940), Bendixen failed to infect rabbits. Daubney and Hudson (1936) working in Kenya, however, and using unfiltered inocula, have reported successful transmission to rabbits (in six cases with brain tissue inoculated subdurally, and in one with defibrinated blood intraperitoneally), and they were able to maintain the virus for a considerable period by passage. These workers also succeeded in passing the virus back from rabbits to cattle, and they stated that they had infected mice and guinea-pigs with their virulent "head-and-eye" strain.

In Palestine attempts were made to infect rabbits, guinea-pigs, rats and mice. These transmission experiments were carried out with filtered and unfiltered material.

*Filtered Inocula.* Inoculations were carried out with material from two fatal cases of malignant catarrh, as follows: —

| *Animal* | *Inoculum* |
|---|---|
| | A Berkefeld N filtrate of brain emulsion in normal saline. |
| 6 rabbits | 0·4 ml. intracranially |
| 4 guinea-pigs | 0·3 ml. „ |
| 4 mice | 0·1 ml. „ |
| | A Berkefeld N filtrate of a saline emulsion of occipital cortex preserved for eight days in 50 per cent. glycerol-saline. |
| 2 rabbits | 0·4 ml. intracranially |
| 1 rabbit | 2·0 ml. intraperitoneally<br>0·4 ml. intracranially<br>2·0 ml. subcutaneously |
| 2 rats | 2·0 ml. intraperitoneally |
| 2 mice | 0·1 ml. intracranially |

These animals were under observation for a maximum of 72 days, and none became affected with malignant catarrh in recognisable form.

*Unfiltered Inocula.* Inoculations were carried out with material from three different fatal cases of malignant catarrh and one non-fatal case, as follows:

| *Animal* | *Inoculum* |
|---|---|
| | Brain Emulsion |
| 3 rabbits | 0·4 ml. intracranially |
| | Whole blood drawn on the 7th day of the temperature reaction and preserved for two days in 50 per cent. glycerol—2 per cent. sodium citrate saline (non-fatal case). |
| 2 rabbits (one—No. 11 —developed malignant catarrh) | 5·0 ml. intraperitoneally |
| | Whole blood drawn on the second and third days of the temperature reaction and preserved for three days in 50 per cent. glycerol—4 per cent. sodium citrate saline. |
| 5 rabbits (one—No. 56 —developed malignant catarrh) | 5·0 ml. intraperitoneally |
| | Whole blood drawn on the third day of the temperature reaction and preserved for one day in 50 per cent. glycerol—4 per cent. sodium citrate saline. |
| 2 rabbits (one—No. 61 —developed malignant catarrh) | 5·0 ml. intraperitoneally |

From these data it will be seen that three rabbits of nine, all of which were inoculated intraperitoneally with citrated whole blood drawn from the affected bovine during the febrile period of the disease developed malignant catarrh. As manifestations of the disease were somewhat different in each of the three affected rabbits, each animal is treated separately.

### THE DISEASE IN RABBITS

*Clinical symptoms and Post-mortem examinations*

*Rabbit No.* 11. No abnormality was noted for 31 days after inoculation. On the 32nd day the temperature was 107·4, and on the 33rd day reached 105·9 with the animal dull, stupid, not eating, and showing a tendency to turn the head to the left and to fall over on that side; corneal reflexes were slow, and movements of the head and fore-limbs sluggish. Two days later a slight improvement was noted; the temperature was 101·8. During the next three days little change occurred. On the 39th day (seven days after symptoms were first noted) there was a sudden change for the worse—head twisted to the right and tendency to turn to the left. The animal was sleepy and bilateral nystagmus was prominent; it was destroyed *in extremis*.

At post-mortem the only abnormalities noted in the viscera were a few white spots on the liver suggesting coccidiosis, and a few small tapeworm cysts in the mesentery. The brain was markedly abnormal showing a large cavity on the right side filled with greyish, watery material, so that almost the entire hippocampal cortex was obliterated. The right side of the brain was enlarged, and the left distorted by pressure. Meningeal haemorrhage was present in the mid-dorsal region overlying part of the cerebrum and cerebellum. The meningeal vessels were somewhat congested. No micro-organism was isolated from heart, lung, liver, spleen, kidney, femur or brain emulsion.

*Rabbit No.* 56. The first signs of disease were noted on the tenth day after inoculation. The temperature was 105·6 (average normal for this animal 103.4) and the right fore-leg extended limply forwards and outwards. On the following day no change was seen. On 12th day there was a slight swelling of the right eyelid with a temperature of 104·8. During the next 20 days there was very little change and no temperature above 104.0 was recorded. On the 31st day the right eye showed a slight, whitish discharge which had become thick by the 32nd day; the eyelid was swollen, and conjunctivitis was present; temperature 105·0. During the next four days little change occurred; the affected limb seemed to be improving and temperatures within the normal range were recorded. Thereafter there was a gradual improvement of both leg and eye until the 67th day when the animal—now apparently normal—was destroyed for examination by the intravenous inoculation of a saturated solution of magnesium sulphate. The only abnormalities noted were a slight paleness of the kidneys and occasional whitish streaks on the liver.

*Rabbit No.* 61. No abnormality was noted for 41 days after inoculation. On the 42nd day the animal was dull and remained so for four days; temperatures between 102·0 and 103·0 were recorded (average normal for this animal 103·8). On the 46th day the animal

was found dead. No abnormality was noted in the viscera other than occasional tapeworm cysts in the mesentery. Two haemorrhages were found overlying the dorsal surface of the cerebrum, just anterior to the fissure separating the cerebrum from the cerebellum, one (the larger) on the right side of the median fissure, the other on the left. Beneath each blood clot the surface of the cerebrum was indented, the larger depression being about 4mm. across and 2 mm. deep.

*Histological examination*

*Rabbit No.* 11. Liver grossly abnormal. Generalised granular degeneration of liver cells; great enlargement of many portal systems, with enlarged bile ducts and dilated blood vessels; loss of normal lobular structure Here and there lesions of granulomatous type, with homogeneous, eosinophilic material in the centre, then a zone of small, round cells tightly packed, then loosely packed fibrous and cellular tissue with numerous multinuclear giant cells (some very large, *e.g.,* 44 nuclei counted), and outside this again liver tissue. In places there were, also, small areas of fibro-cellular tissue, possibly an early stage of the graulomatous lesions just described.

Kidney. Some of the glomeruli slightly swollen and somewhat distorted and, under low magnification, apparently merging with the surrounding tubular tissue.

Lung. Slight, patchy oedema and some thickening of the interalveolar tissue. One small haemorrhage noted.

Prescapular lymphatic gland. No abnormality detected.

Frontal cortex. Well marked, tight vascular cuffing with cells apparently lymphocytes and larger mononuclears; no polymorph seen; infiltrating cells almost invariably confined to perivascular lymph channels. Numerous degenerating nerve cells in grey matter; neuronophagia noted. Cell infiltration in the tissue localised round left lateral ventricle. Infiltration round some meningeal vessels.

Section of macroscopic lesion. Lesion a large cavity in hippocampal cortex; cavity mostly empty but round the edges masses of cells (mostly polymorphs, together with other cells with large, pale, rather eosinophilic, vesicular nuclei, and lymphocytes) and cell debris. Lining the cavity a closely packed mass of cell and cell debris, and outside, both medially and laterally, intense tissue reaction with vascular and perivascular cuffing (mainly lymphocytes, together with larger mononuclears, occasional plasma cells, and occasional polymorphs), tissue infiltration and nerve cell damage. Pyramidal cells of hippocampal region mostly degenerating. Neuronophagia noted. Cuffing of some meningeal vessels.

Hippocampal cortex. Extensive damage on side of macroscopic lesion. On the other side cuffing of occasional vessels. No evidence of damage to pyramidal cells.

Mid-brain. Cuffing of some meningeal vessels. Little other abnormality apart from an area showing vascular infiltration and tissue infiltration close to macroscopic lesion.

Cerebellum. Marked meningeal reaction, the inflammatory cells being lymphocytes, plasma cells, large mononuclears, and polymorphs. Slight cuffing of occasional vessels.

Pons. Little apparent abnormality.

Medulla. Slight vascular infiltration of occasional small vessels in

ventral region. Meningeal reaction visible. Little apparent abnormality of nerve cells.

Cervical cord. Slight degenerative changes in occasional nerve cells. The most intense inflammatory reaction in the brain substance appeared to be related to the meningitis, and in the anterior part of the brain to the tissue surrounding the ventricles.

*Rabbit* No. 56. Liver. Fairly marked degeneration of hepatic cells, with nuclei pyknotic and cytoplasm showing fatty degeneration and granulation. Slight, but quite definite, cellular infiltration (mainly lymphocytes, occasional larger mononuclears, and rare plasma cells) into Glisson's capsule.

Kidney. Small accumulations of homogeneous material staining pink with eosin within Bowman's capsule in some of the glomeruli; clear spaces in some of the glomerular tufts.

Spleen. Some Malpighian bodies unusually prominent.

Lung and Brain. No abnormality detected.

*Rabbit No.* 61. Liver. No apparent abnormality of hepatic cells. Slight, but quite definite, cellular infiltration (lymphocytes and larger mononuclears) into Glisson's capsule, and to a small extent outside the capsule. Sometimes infiltrating cells tightly packed round bile-ducts, in other cases spread along the side of both vessels and bile-ducts. Slight infiltration occasionally seen round a central vein. One focus of lymphocytes noted apparently unconnected with any vessels. Many Küpffer cells apparently swollen. A good deal of blood in the sinusoids.

Heart. No abnormality detected.

Frontal cortex. Occasional nerve cells showing slight degenerative changes; one definite area of focal cell infiltration deep in the grey matter.

Parietal cortex. Occasional nerve cells showing slight degenerative changes; no apparent microscopical reaction beneath the macroscopical depression (q.v.). One small vessel showing perivascular cuffing with local cellular infiltration of the tissue surrounding it.

Mid-brain. Occasional nerve cells showing slight degenerative changes; one big focus of cell infiltration into the brain tissue.

Olfactory bulb; hippocampal cortex; pons: medulla: cervical cord. Rare nerve cells showing slight degenerative changes.

*Subinoculations*

Subinoculations were made only from Rabbit No. 11, as follows:

| *Animal* | *Inoculum* |
|---|---|
| Rabbit No. 44 (Died after 34 days) | 2·0 ml. whole blood intraperitoneally |
| Rabbit No. 45 (Destroyed after 77 days) | 0·4 ml. brain suspension intracranially. |
| Rabbit No. 46 (Died after 32 days) | 0·25 ml. brain suspension intracranially |

Of these animals, Nos. 44 and 46 are believed to have been affected with malignant catarrh. The other (No. 45) which became ill 77 days after inoculation, was destroyed, and was found affected with multiple abscesses of all organs; a haemolytic staphylococcus was isolated in pure culture from the heart and liver.

The following are brief notes on the two rabbits—Nos. 44 and 46—subinoculated from rabbit No. 11.

*Rabbit No.* 44. Thirty-three days after inoculation the animal appeared to be dull, the abdomen was tense, and the temperature was 99·8 (average normal for this animal 103·4). Death occurred during the night of 33rd day. Post-mortem examination showed some distension of the stomach, congestion of the lungs, excess of dark peritoneal fluid, congestion of the meningeal vessels running over the cerebellum and ventral surface of the pons and medulla. Bacterial culture revealed a variety of organisms all of which were accepted as putrefactive in type. In the liver histological examination showed marked enlargement of bile-ducts, together with proliferation of bile-duct epithelium, and occasional cell infiltration (lymphocyte, plasma cell, and larger mononuclear) into Glisson's capsule. In places there was quite marked degeneration of liver cells, especially in the vicinity of the central veins; the cyptoplasm of the liver cells was markedly granular. In the kidney some glomerular and capillary congestion was observed. No abnormality was detected in the brain, but occasional apparently degenerating nerve cells were noted among the ventral horn cells of the cervical cord.

Subinoculations of unfiltered brain/liver emulsion intraperitoneally in one rabbit and four mice failed to produce evidence of disease.

*Rabbit No.* 46. No clinical symptoms were noted until 31 days after inoculation. On that day the temperature dropped to 99·6 (average normal for this animal 103·8) and the animal was dull and uninterested in food. On the following morning it was shivering, dull and the abdomen was distended. There was a slight shaking of the head, and at rest the left fore-limb was stuck forwards. The temperature fell to 97·0 and the animal died shortly after noon. Just before death a semi-fluid, amber-coloured discharge came from the left nostril. Post-mortem examination revealed occasional white spots on the liver (confirmed histologically as coccidiosis), distension of the stomach with a few tiny ulcers in the mucosa, and congestion of the meningeal vessels. No bacterial growth was obtained from the liver and heart. Histological examination of the liver showed coccidiosis and another lesion, similar to that described in the liver of rabbit No. 11, *viz.*, a pinkish, homogeneous material in the centre with cells scattered through it, and outside this an area of loose, apparently fibrous tssue, then another ring of tightly packed cells, and finally liver tissue. In this case many of the reactionary cells were eosinophils. In the kidney there was some swelling of the glomeruli. No abnormality was detected in the brain or cervical cord.

Subinoculations of unfiltered brain emulsion and filtered liver/spleen emulsion intracranially in three rabbits failed to produce evidence of disease.

### DISCUSSION OF RESULTS

Daubney and Hudson (1946) have described fully their successful infection of rabbits with the virus of bovine malignant catarrh and comparison of their findings with those in Palestine shows a close similarity between the diseases in the two countries.

Histological findings in rabbits in Palestine have been described in some detail, and they indicate that the animals were successfully infected with the virus of bovine malignant catarrh. Additional evidence of successful transmission is afforded by the meningeal haemorrhages noted in rabbits Nos. 11 and 61, and by the death of two rabbits (Nos. 44 and 46) subinoculated from rabbit No. 11. The fact that transmission was so irregular appears also to be of significance.

### HISTOLOGICAL FINDINGS IN CASES OF THE DISEASE IN CATTLE

A few authors (*e.g.*, Mettam, 1923; Dobberstein, 1925; Bisbocci, 1934; Daubney and Hudson, 1936; Hutyra, Marek and Manninger, 1938; Schofield and Bain, 1941) have given accounts of the histopathological findings in bovine malignant catarrh but although their findings largely agree with those in Palestine, sufficiently marked differences were noted to make a detailed description of Palestinian findings worth while.

#### *Material Examined*

The material available for histological examination was obtained from eight fatal cases of "head-and-eye" malignant catarrh.

### HISTOLOGICAL FINDINGS

(The figure in brackets indicates the number of different animals from which the particular tissue was obtained.)

*Liver* (5). The most marked abnormality noted in the liver was an infiltration into Glisson's capsule of lymphocytes, larger mononuclears, plasma cells, occasional eosinophils, and rare polymorphs. In one case this invasion was extensive while in the other four, although present, it was far less prominent. It was noted that sometimes when infiltration was slight the cells were grouped round bile-ducts, leaving the blood vessels entirely free. The infiltrating cells were usually, but not invariably, confined within Glisson's capsule. Infiltration round a central vein was rare, and was noted only three times in all the sections examined, and even so was only slight. Occasional foci of cells apparently unrelated to any vessel were also noted. Degenerative changes in the liver cells were only slight and mainly of the nature of cloudy swelling, most noticeable in cells at the centre of lobules; in one case some loss of nuclear outline was noted in cells in this region. Slight fatty degeneration of liver cells was observed in three cases. Swelling of Küpffer cells was noted in three cases, and in one case many of the sinusoids were distended

with blood. Congestion and rare haemorrhages of subcapsular vessels were seen in one case.

These findings agree fairly closely with those described by other observers. Mettam (1923) noted fatty degeneration of liver cells, together with lymphocytic infiltration into the portal systems, and Daubney and Hudson (1936) say, "The infiltrating cells surround artery, vein and bile-duct in the portal canal; they may be wholly confined within the greatly distended Glisson's capsule or at points may escape into the lobule proper."

It may be worth drawing attention to the observation made above that cases were observed in Palestine where the infiltrating cells were clustered only round the bile-duct.

*Kidney* (5). In all cases aggregations of infiltrating cells (lymphocytes larger mononuclears, and occasional plasma cells) were frequently related to a blood vessel, or surrounded a glomerulus, but in some cases a perivascular distribution could not be demonstrated. The cellular infiltrations were noted in all regions of the kidney, but were perhaps most commonly found in the cortex. Not by any means every visible glomerulus or blood vessel had infiltrating cells related to it. In general, the glomeruli appeared to be normal, but sometimes were slightly swollen, and in some cases a number of shrunken glomeruli was noted. In two cases a homogeneous material staining pink with eosin was present inside Bowman's capsule. Occasional small haemorrhages were noted in two cases. Very little abnormality of the kidney parenchyma was noted in any case, and at most amounted only to cloudy swelling of tubular epithelium. The "pressure necrosis" of tubular epithelium described by Mettam (1923) and by Daubney and Hudson (1936) in the region of cell infiltrations was not observed, although in some areas the aggregations of infiltrating cells obliterated the tubular structure. In one case a few small casts were noted in collecting tubules in the pelvic region.

Again these findings agree fairly closely with those described by other workers, although the cell infiltration observed by Mettam and by Daubney and Hudson would appear to have been more marked than in the cases observed in Palestine. Bisbocci (1934) noted a well-marked connective tissue reaction, hyperaemia and shrinking of the glomeruli in all his cases and, commonly, areas of infiltration in the neighbourhood of glomeruli, and desquamating cells in the capsular space. In some more chronic cases of the disease, Daubney and Hudson (1936) observed small cysts devoid of epithelial lining and formed by the coalescence of two or three tubules.

*Spleen* (4). No very marked abnormality was noted. Slight perivascular infiltration of rare vessels in the trabeculae was observed in two cases, and slight subcapsular haemorrhage in one case. In general, the normal structure of the spleen was well preserved. Mettam (1923) reported mitotic figures in the lymphocytic follicles, colonies of lymphocytes near small blood vessels of the

capsule and trabeculae and a large amount of blood within the organ. *Lymphatic glands* (6). In one case there was marked patchy haemorrhage involving both cortex and medulla, and in four cases the gland appeared fully active. In only one case could mitotic figures be found, and then only occasionally. In three cases perivascular infiltration of occasional vessels was seen. In one case there appeared to be slight subcapsular oedema. Mettam (1923) noted that the germinal centres in lymphatic glands were overcrowded with numerous lymphocytes. Daubney and Hudson (1936) speak of congestion, oedema, pericascular infiltration of vessels in the trabeculae, and activity in many of the germinal centres.

*Brain and cervical cord.* The non-purulent encephalitis reported by Dobberstein (1925) was present in all eight brains examined. In some cases it was accompanied by a meningitis, probably secondary in nature. Detailed findings are as follows:—

*Frontal cortex* (3). In all three cases vascular cuffing of numerous vessels in both the substance of the brain and meninges was prominent. The infiltrating cells were apparently lymphocytes, larger mononuclears, and occasional plasma cells; in the vicinity of vessels in the substance of the brain these cells were usually, but not invariably, confined within the perivascular lymph channels in the form of a tight cuff. In two cases the meningeal reaction was slight, but in the other well-marked, with a considerable aggregation of cells between the pia mater and the surface of the brain, in some cases infiltrating into the brain substance itself. In one case the nerve cells were apparently undamaged but in the two others early degenerative changes were noted in many cells, amounting to loss of cell outline and "irregularity" of cytoplasm. Neuronophagia was occasionally observed, and an apparent increase in satellitism. In all cases there were areas of focal cellular infiltration (apparently unconnected with any blood vessel) into both grey and white matter.

*Parietal cortex* (6). In all cases cellular infiltration of many vessels in both grey and white matter was visible, and in rare cases infiltration into the brain substance beyond the perivascular lymph channel was noted. Infiltrating cells were of the same type as seen in the frontal cortex. Meningeal reaction was marked in one case, slight in four cases, and apparently absent in the other. Occasional foci of infiltrating cells in the brain substance were noted. In general, the nerve cells appeared to be undamaged, but occasional cells showing slight degenerative changes were found. Occasional small haemorrhages in the region of blood vessels were noted in one case.

*Occipital cortex* (4) Vascular infiltration was obvious in all cases. and meningeal reaction in three. There were occasional small areas of focal cell infiltration into the brain substance. Only slight degenerative changes were seen in occasional nerve cells.

*Hippocampal cortex* (5). Histological picture as for parietal cortex. Meningeal reaction noted in four of the five cases.

*Basal ganglia* (3). There was obvious vascular and perivascular infiltration in all cases, and areas of focal cellular infiltration in both grey and white matter. Only slight degenerative changes were noted in occasional nerve cells.

*Mid-brain* (4). Well-marked vascular and perivascular infiltration was noted in all cases; also occasional areas of focal cell infiltration in the brain substance. In three cases occasional nerve cells showed slight degenerative changes, and in the other case slightly degenerated cells were fairly numerous .

*Pons* (5). There was a well-marked vascular and perivascular infiltration with areas of focal cell infiltration, and occasional nerve cells showing slight degenerative changes.

*Medulla* (5). Same general changes were observed as in the pons, with infiltration into the perivascular lymph channels prominent. In one case numerous nerve cells apparently undergoing degenerative changes were noted.

*Cerebellum* (4) and *cervical cord* (2). Vascular and perivascular infiltration of numerous vessels and areas of focal cell infiltration were evident.

Note.—Apparent dilatation of some blood vessels was noted in various regions of the brain.

### DISCUSSION OF FINDINGS IN THE CENTRAL NERVOUS SYSTEM

As has been pointed out elsewhere (Pattison, 1945) the question of the significance of slight apparently degenerative changes in nerve cells in the central nervous system of animals, as seen in many sections prepared from cases of bovine malignant catarrh, is one that is very dfficult to answer. In many sections prepared from different domestic and laboratory animals affected with a wide variety of diseases, and some apparently healthy when destroyed, the writer has observed occasional nerve cells that according to text-book descriptions would appear to be undergoing degenerative changes. The purkinje cells of the cerebellum are an outstanding example. It is felt, therefore, that until the significance of these changes is fully understood it would be unwise to lay much stress on their importance. The possible presence of inclusion bodies in nerve cells was considered, and these were searched for with negative results.

Mettam (1923) found " lymphocyte foci of various sizes " about the blood vessels of the brain and spinal cord, and a " slight increase of the number of lymphocytes . . . in the substance of the brain, but always immediately beside small capillaries. A similar condition was observed in the epineurium and perineurium of many of the peripheral nerves, but in no case were any degenerative changes noted in the actual nervous tissue." Bisbocci (1934) noted masses of cells, mainly lymphocytes, between the arachnoid and pia mater, perivascular infiltration in various parts of the cerebral cortex (lymphocytes and small plasma cells), and areas of degeneration and necrosis

in the stratum lucidum and other parts of the brain. Daubney and Hudson (1936) reported, as an almost constant feature in their cases, cuffing of vessels in both grey and white matter of the brain and spinal cord; in some cases the infiltrating cells were not confined within the Virchow-Robin space. They state, "In some instances we have found what appear to be purely focal infiltrations, generally in the grey matter, and occasionally satellitism of neurons has been in evidence". These observers were unable to discover Joest-Degen corpuscles. Mutyra, Marek and Manninger (1938) have stated that in bovine malignant catarrh the vascular and perivascular infiltrations of lymphocytes and plasma cells are more pronounced in the cerebral cortex, basal ganglia and mid-brain. They say that the histological picture resembles that seen in Borna disease.

With regard to these observations made by other workers, it may be noted that in Palestine focal tissue infiltrations undoubtedly unconnected with blood vessels were found in both grey and white matter, that no large area of degeneration was found, that there was no constant evidence that the inflammatory reaction was more intense in the cerebral cortex, basal ganglia, and mid-brain than in other regions, and that although the histological picture in the brain undoubtedly resembled descriptions of Borna disease, it also resembled findings noted by the writer in individual cases of equine encephalomyelitis, ovine encephalitis, and swine fever. It was, in fact, in the writer's opinion merely a non-purulent encephalitis with no outstanding feature by which it could readily be differentiated from the type of non-purulent encephalitis that may be encountered in other diseases.

## SUMMARY

Details are given of attempts to infect rabbits, guinea-pigs, rats, and mice with the virus of bovine malignant catarrh. Filtered and unfiltered inocula were used, and it is believed that three rabbits were successfully infected by intraperitoneal inoculation of unfiltered, citrated blood.

The disease in rabbits is described, and the conclusion is reached that it is probably of the same nature as that described by Daubney and Hudson (1936) in Kenya.

Histological findings in tissues (liver, kidney, spleen, lymphatic gland, brain, and cervical cord) obtained from eight clinically typical cases of "head-and-eye" bovine malignant catarrh in Palestine are described, and are compared with findings reported by workers in other countries.

It is concluded that the encephalitis noted in all cases was of a non-purulent type with no outstanding feature by which it could readily be differentiated from the type of non-purulent encephalitis found in a variety of other diseases.

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REFERENCES

Aleska. (1935). *Rec. Méd. vét.*, **111**, 274.
Bisbocci, G. (1934). *Nuovo Ercol*, **39**, 281.
Daubney, R., and Hudson, J. R. (1936). *J. comp. Path*, **49**, 63.
Dobberstein, J. (1925). *Dtsch. tierärztl. Wschr..*, **33**, 867.
Dobberstein, J., and Hemmert-Halswick, A. (1928). *Z. InfektKr. Haust.*, **34**, 160.
Götze, R., and Liess, J. (1929). *Dtsch. tierärztl. Wschr.*, **37**, 433 ; (1930). *ibid.*, **38**, 194.
Hutyra, F., Marek, J., and Manninger, R. (1938). *Special Pathology & Therapeutics of the Diseases of Domestic Animals*. 4th ed. London: Baillière, Tindall & Cox.
Magnusson, H. (1940). *Beretn. 5 nord. Vet mode, Kbh.*, 1939. 369.
Mettam, R. W. M. (1923). *9th & 10th. Reps. Dir. Vet. Ed. & Res., S. Africa*. 393.
Miessner, H., and Schoop, G. (1934). *Dtsch. tierärztl. Wschr.*, **42**, 115.
Pattison, I. H. (1945). *J. comp. Path.*, **55**, 109.
Schofield, F. W., and Bain, A. F. (1941). *Canad. J. comp. Med.*, **5**, 294.
Stolnikoff, W. G. (1933). *Z. InfektKr. Haust.*, **43**, 100.
Zanzucchi, A. (1934). *Clin. vet., Milano.*, **57**, 689.

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# THE RATE OF PASSAGE OF FOOD RESIDUES THROUGH THE DIGESTIVE TRACT OF THE HORSE

By

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## INTRODUCTION

In view of the complexity of the equine digestive tract considerable interest has been aroused in the time taken for food residues to traverse it. This phenomenon has been studied in man and the common laboratory animals by a variety of methods, but, excepting the observation of Fish (1923), no similar studies appear to have been carried out on the horse. The methods so far employed have depended on the addition of small coloured beads to the food (Elliot and Barclay-Smith, 1904), the admixture of an inert insoluble colouring matter (Guernsey and Evvard, 1913), or roentgenological studies

Table I

The Faecal Excretion of Ingested Carbon Granules

| *Horse* | *Observation No.* | *Time of appearance of indicator in faeces* | |
|---|---|---|---|
| | | *Beginning* | *End* |
| Chestnut mare ... | 1 | 18·5 hours | 44·5 hours |
| | 2 | 19·0 „ | |
| | 3 | 25·0 „ | 58·0 „ |
| | 4 | 22·0 „ | 44·0 „ |
| | 5 | 23·0 „ | 39·0 „ |
| | Mean | 21·5 „ | 46·4 „ |
| Bay mare ... ... | 1 | 18·5 hours | 68·5 hours |
| | 2 | 18·0 „ | 50·0 „ |
| | 3 | 44·0 „ | 67·0 „ |
| | 4 | 22·5 „ | 40·0 „ |
| | 5 | 18·0 „ | 40·0 „ |
| | 6 | 22·0 „ | 44·0 „ |
| | Mean | 23·8 „ | 51·6 „ |
| Bay mare (2) ... | 1 | 21·0 hours | 48·0 hours |
| Black mare ... | 1 | 23·0 hours | 47·0 hours |
| | 2 | 23·0 „ | 55·0 „ |
| | 3 | 24·0 „ | 46·0 „ |
| | 4 | 21·0 „ | 39·0 „ |
| | 5 | 21·5 „ | 39·5 „ |
| | Mean | 22·5 „ | 45·3 „ |
| Chestnut gelding ... | 1 | 22·0 hours | 37·0 hours |
| *Mean of all observations* ... ... ... | | 22·6 ± 1·32 (S.E.) | 47·4 ± 2·26 (S.E.) |

of the rate of passage through the gut of a barium meal. In the experiments described here carbon particles were mixed with the food and the rate of their excretion in the faeces observed.

## METHODS

The method employed in these experiments was to keep the animals on a maintenance diet of oats 6 lb., bran 2 lb., and hay 12 lb. One hundred gm. of granular carbon were mixed with a normal feed of oats and bran and the time of feeding noted. All faeces passed after this feed were carefully examined for the presence of carbon. The granules used were of about the size of a mustard seed and could easily be seen with the naked eye. The examination was facilitated by shaking the faeces with water in a cylinder when much of the fibrous matter floated off.

Carmine, ferric oxide, kaolin and beads were tried as markers but proved unsatisfactory. The first three substances were difficult to detect, whilst the last was not recovered even by the end of one week.

## RESULTS

The results of these experiments are tabulated in Table 1.

## DISCUSSION

The paucity of data about the progress of food residues through the equine digestive tract prompted further investigation, using granules of carbon as the indicator. This substance fulfilled the criteria of Alvarez (1940) in that it had little bulk compared to the total amount of the meal, it mixed intimately with the usual food, and was easy to detect. Furthermore, in view of the observations of Hoelzel (1930) that substances with a high specific gravity tend to lag behind the food residue, it was important to choose a material having a density similar to the normal food constituents. It was found that granular carbon met this requirement. Roentgenological studies were, of course, impracticable.

The results of these experiments show that the time taken for the food residue of a normal meal to traverse the equine digestive tract is approximately the same as Burnett (1923) and Alvarez (1940) have found for man. In view of the great disparity between the human and equine digestive tracts in both their relative lengths and complexities this observation is striking and the explanation not obvious.

## SUMMARY

Carbon granules mixed in a feed of oats and bran and fed to horses appeared in the faeces in 22·6 (±1·32) hours and continued up to 47·4 (±2·26) hours after feeding.

The figures were the means of seventeen seperate observations.

REFERENCES

Alvarez, W. C. (1940). *An Introduction to Gastro-Enterology.* 3rd Ed.
Burnett, F. L. (1923). *Am. J. Roentgenol.*, **10,** 105.
Elliot, T. R., and Barclay-Smith, E. (1904). *J. Physiol.*, **31,** 272.
Fish, P. A. (1923). *Proc. Soc. exp. Biol. and Med.*, **20,** 524.
Guernsey, S. C., and Evvard, J. M. (1913). *Biochem. Bull,* **3,** 369.
Hoelzel, F. (1930). *Am. J. Physiol.*, **92,** 466.

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# THE INCIDENCE OF BLACK-QUARTER IN NATIVE CATTLE IN THE CENTRAL PROVINCE, NYASALAND

By

S. G. Wilson

## INTRODUCTION

Black-quarter disease in cattle is usually caused by the spore-forming soil, anaerobic bacillus *Clostridium chauvoei*, and more rarely by *Cl. septique*.

No previous record could be found of *Cl. chauvoei* infections being diagnosed in the Central Province of Nyasaland. Smith (1931) records it from Northern Rhodesia where, although frequently reported, no heavy losses occurred. Hornby (1933) reports a few outbreaks from Tanganyika where mortality was serious,and a Kabete vaccine proved satisfactory. No data is given from either of these territories to show the exact mortality that occurred. No records can be traced of the disease having been diagnosed in Portuguese East Africa.

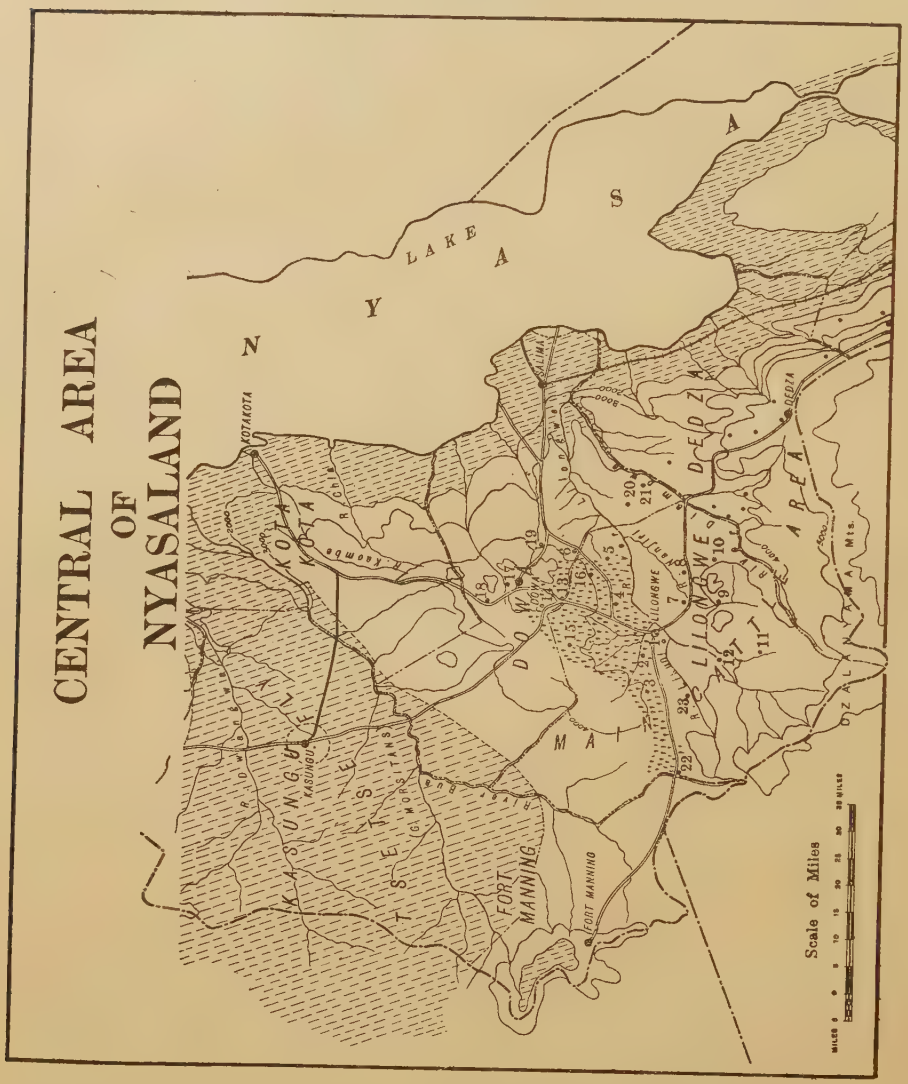
## DIAGNOSIS AND GEOGRAPHICAL DISTRIBUTION OF THE DISEASE IN NYASALAND DURING 1944

On October 25th, 1944, reports of a relatively heavy mortality in young animals at Likuni tank area were investigated. Five beasts showing clinical signs of black-quarter and the carcase of seven other young animals which had recently died were examined, and there was no hesitation in diagnosing black-quarter as being the cause of the epizootic. Smears from affected muscle showed bacilli indistinguishable from *Cl. chauvoei* and exudate from swellings injected into three experimental animals caused death in from 48 to 60 hours. The disease at this tank area remained localised in about ten adjacent villages. Subsequent investigations, however, revealed similar outbreaks at nine other dipping tank areas. The exact distribution of the disease in 1944 is shown in Map I. Cattle in the tank areas south and east of the infected area remained clear of the disease.

## GENERAL DISEASE MORTALITY IN THIS AREA

Exact data on the total mortality from all bovine diseases in the Central Province of Nyasaland have been accumulated at Lilongwe since 1942. Previous to that year cattle owners were only obliged to report deaths in their herds if a scheduled disease was suspected and, as a result, the Veterinary Department possessed no accurate vital statistics. In 1942, legislation was passed compelling cattle owners in the dipping tank areas of the Central Province to report all changes in the numbers of cattle owned due to deaths, births and movements. Records were kept on stock cards given to each cattle owner and summarised on cattle registers kept by the trained African

Veterinary Assistants in charge of the nearest dipping tank. The accuracy of the system was ensured by frequent detailed inspections by the Veterinary Officer. When any of his cattle died the owner was required to report each case within 24 hours to the Veterinary




Assistant of his tank area, and to submit a spleen from each animal. The Veterinary Assistant then made whatever investigation was considered necessary and recorded in his cattle register the date age and sex of the beast and the cause of death. A spleen smear was also forwarded with a detailed smear form in duplicate to Lilongwe

laboratory. Other changes in the herd were similarly recorded in the cattle register and from the constant checking and study of the accumulated data, the Veterinary Officer became familiar with the general trend of changes occurring in this cattle population where the system of animal management and climatic conditions varied but slightly from place to place. The most obvious seasonal changes that occurred are shown in Table I and Fig. 1. The area chosen was designated "Group C" tank area and consisted of eight tank areas during January and February, 1943, and nine tank areas during the remaining months from March, 1943, onwards.

TABLE I

STATISTICS FROM GROUP "C" TANK AREA SHOWING CATTLE POPULATION, BIRTH RATE AND DEATH RATE FROM DISEASE IN 1943-45

| | | *Cattle population 1st of month* | *Calves born* | *Calving rate per 1,000* | *Deaths from disease* | *Deaths from disease per 1,000* |
|---|---|---|---|---|---|---|
| 1943 | Jan. | 23,194 | 163 | 7·0 | 84 | 3·6 |
| | Feb. | 22,522 | 304 | 13·5 | 240 | 10·6 |
| | Mar. | 24,871 | 231 | 9·2 | 373 | 14·9 |
| | Apr. | 24,142 | 307 | 12·7 | 108 | 4·4 |
| | May | 23,668 | 230 | 9·7 | 88 | 3·7 |
| | June | 23,717 | 957 | 40·3 | 72 | 3·0 |
| | July | 22,618 | 959 | 43·7 | 55 | 2·4 |
| | Aug. | 24,830 | 1,072 | 43·1 | 49 | 1·9 |
| | Sept. | 25,386 | 969 | 38·1 | 28 | 1·1 |
| | Oct. | 25 943 | 775 | 29·8 | 49 | 1·8 |
| | Nov. | 26,142 | 649 | 24·4 | 131 | 5·0 |
| | Dec. | 25,899 | 327 | 12·5 | 215 | 8·3 |
| 1944 | Jan. | 25,459 | 212 | 8·0 | 442 | 13·4 |
| | Feb. | 24,575 | 272 | 11·0 | 474 | 19·2 |
| | Mar. | 24,025 | 271 | 11·2 | 404 | 16·3 |
| | Apr. | 23,426 | 318 | 13·5 | 198 | 8·4 |
| | May | 23,251 | 529 | 22·7 | 110 | 4·7 |
| | June | 23,298 | 905 | 38·8 | 129 | 5·5 |
| | July | 23,663 | 1,362 | 57·5 | 93 | 3·9 |
| | Aug. | 24,285 | 1,440 | 59·2 | 88 | 3·6 |
| | Sept. | 25,035 | 963 | 38·4 | 78 | 3·2 |
| | Oct. | 25,360 | 899 | 35·4 | 57 | 2·2 |
| | Nov. | 25,796 | 624 | 24·1 | 102 | 3·9 |
| | Dec. | 25,910 | 250 | 9·6 | 136 | 5·2 |
| 1945 | Jan. | 25,543 | 348 | 15·0 | 308 | 12·1 |
| | Feb. | 25,147 | 207 | 8·2 | 406 | 16·1 |
| | Mar. | 24,539 | 235 | 9·6 | 419 | 17·1 |
| | Apr. | 23,859 | 253 | 10·6 | 309 | 12·9 |
| | May | 23,551 | 335 | 14·3 | 134 | 5·7 |
| | June | 23,460 | 607 | 25·4 | 144 | 6·1 |
| | July | 23,530 | 1,133 | 48·0 | 211 | 8·96 |
| | Aug. | 23,862 | 866 | 36·4 | 110 | 4·6 |
| | Sept. | 24,208 | 820 | 33·9 | 73 | 3·2 |
| | Oct. | 24,053 | 766 | 32·0 | 84 | 3·5 |
| | Nov. | 24,551 | 406 | 16·6 | 106 | 4·3 |
| | Dec. | 24,590 | 288 | 11·7 | 128 | 5·2 |

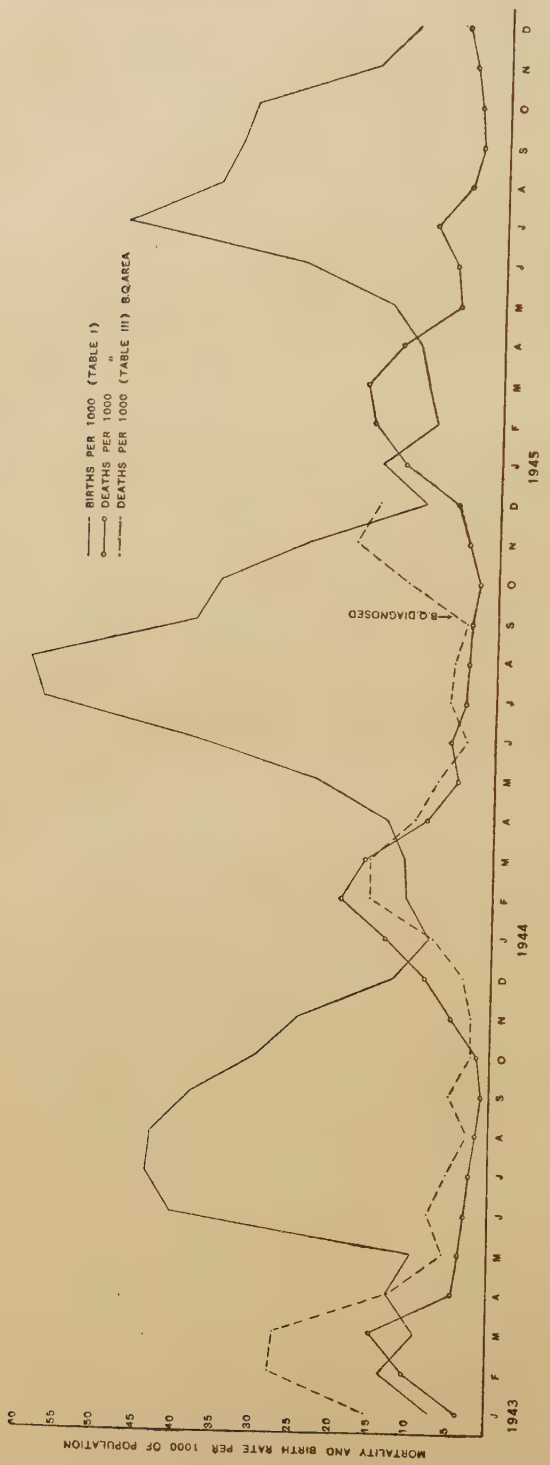

BIRTHS PER 1000 (TABLE I)
DEATHS PER 1000 "
DEATHS PER 1000 (TABLE III) B.Q.AREA
B.Q. DIAGNOSED
MORTALITY AND BIRTH RATE PER 1000 OF POPULATION
60
55
50
45
40
35
30
25
20
15
10
5
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1943
1944
1945

FIG 1

From a study of Table I, and from similar data collected in other areas it became evident that the incidence of disease showed a definite seasonal prevalence. During the wet hot months, from December to April, mortality from disease was over five deaths per 1,000 head of cattle per month, reaching a peak in February or March when deaths were 15 per 1,000 or more. During the dry months of May to November mortality was usually under five per 1,000 per month. The birth curve showed an opposite tendency, the calving rate being high during the cold dry months of June to October and low during the rainy months of December to April. Any deviation from these prevailing tendencies was, therefore, the subject of immediate investigation.

### ESTIMATION OF THE MORTALITY DUE TO BLACK-QUARTER

Exact data for the mortality due to black-quarter alone was only collected for a few small areas. The present survey was especially designed to obtained precise figures for the incidence of protozoan diseases and spleen smears were being collected from all dead animals in the area. In native areas such as we are dealing with, it is not easy suddenly to change the procedure of diagnosis. Nor can the Veterinary Officer or his assistants make a clinical diagnosis in every infected herd owing partly to pressure of other duties and partly to the nature of the country where communications are slow. Moreover, the reports as to exact cause of death submitted by Veterinary Assistants and cattle owners cannot be accepted as accurate in every case. In most cases, therefore, an estimate of the extent of the black-quarter outbreaks has been calculated by comparing the mortality in the infected area with that occurring in adjacent regions where black-quarter was absent, *e.g.*, areas such as those

TABLE II

DISEASE MORTALITY RATE FOR LIKUNI TANK AREA DURING 1943–1945

| | 1943 | | | 1944 | | | 1945 | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Cattle population* | *Deaths from disease* | *Deaths from disease per* 1,000 | *Cattle population* | *Deaths from disease* | *Deaths from disease per* 1,000 | *Cattle population* | *Total deaths from disease* | *Deaths from disease per* 1,000 |
| Jan. | 4,019 | 47 | 11·5 | 3,725 | 36 | 9·5 | 3,503 | 59 | 16·8 |
| Feb. | 3,816 | 106 | 27·7 | 3,671 | 74 | 20·2 | 3,424 | 78 | 22·8 |
| Mar. | 3,810 | 90 | 23·6 | 3,548 | 60 | 16·9 | 3,323 | 45 | 13·5 |
| Apr. | 3,728 | 40 | 10·5 | 3,303 | 43 | 13·0 | 3,252 | 46 | 14·1 |
| May | 3,649 | 39 | 10·7 | 3,210 | 20 | 5·6 | 3,212 | 21 | 6·5 |
| June | 3,681 | 42 | 11·4 | 3,218 | 21 | 6·5 | 3,189 | 21 | 6·6 |
| July | 3,594 | 43 | 11·9 | 3,272 | 21 | 6·2 | 3,255 | 34 | 10·4 |
| Aug. | 3,652 | 29 | 7·9 | 3,305 | 24 | 7·3 | 3,368 | 24 | 7·1 |
| Sept. | 3,728 | 29 | 7·6 | 3,457 | 13 | 3·8 | 3,471 | 10 | 2·9 |
| Oct. | 3,714 | 18 | 4·8 | 3,518 | 40 | 11·4 | 3,502 | 23 | 6·5 |
| Nov. | 3,753 | 18 | 4·8 | 3,534 | 33 | 9·3 | 3,552 | 11 | 3·1 |
| Dec. | 3,752 | 14 | 3·8 | 3,551 | 24 | 7·1 | 3,546 | 12 | 3·4 |

given in Table I. The results have also been correlated with mortality figures for other seasons when black-quarter was absent.

The effect of the epizootic in the Likuni tank area is shown in Table II. During October, 1944, when black-quarter was prevalent, cattle deaths from all diseases rose from the normal figure of 3·8 head per 1,000 in September to 11·4. By comparison, deaths in October, 1943, in this area were only 4·8 per 1,000. The actual mortality in October, 1944, due to black-quarter was diagnosed as 24 animals or approximately 7 per 1,000 head of cattle population.

The disease mortality for the whole black-quarter area as shown in Map 1 is given in Table III.

TABLE III

MORTALITY IN BLACK-QUARTER AREAS DURING 1943–1944

| | 1943 | | | 1944 | | |
|---|---|---|---|---|---|---|
| | *Cattle population 1st of each month* | *Total deaths from disease* | *Deaths from disease per 1,000* | *Cattle population 1st of each month* | *Total deaths from disease* | *Deaths from disease per 1,000* |
| Jan. ... | 24,813 | 377 | 15·2 | 36,248 | 284 | 7·8 |
| Feb. ... | 26,810 | 744 | 27·7 | 35,685 | 554 | 15·5 |
| Mar. ... | 35,790 | 971 | 27·1 | 36,891 | 575 | 15·6 |
| Apr. ... | 33,286 | 422 | 12·7 | 34,095 | 342 | 10·0 |
| May ... | 34,113 | 198 | 5·8 | 33,679 | 238 | 7·1 |
| June ... | 34,248 | 261 | 7·6 | 33,909 | 199 | 3·5 |
| July ... | 34,573 | 179 | 5·2 | 34,354 | 202 | 5·9 |
| Aug. ... | 35,005 | 100 | 2·8 | 35,047 | 183 | 5·2 |
| Sept. ... | 35,257 | 181 | 5·1 | 35,829 | 137 | 3·8 |
| Oct. ... | 36,066 | 82 | 2·3 | 36,152 | 413 | 11·4 |
| Nov. ... | 36,639 | 84 | 2·3 | 36,584 | 663 | 18·1 |
| Dec. ... | 36,562 | 129 | 3·5 | 36,374 | 550 | 15·1 |
| *Totals* | | 3,728 | 117·3 | | 4,340 | 119·1 |

During January and February, 1943, returns from four tanks and three tanks respectively are not included in Table III. From March, 1943, onwards, the returns are from nine tank areas. Fig. 1 aslo shows the mortality rate per 1,000 head of population of this area (Table III) compared with that from the non-black-quarter districts.

During 1943 the mortality rates for the two areas were very similar, and they remained so until September, 1944. During October, November, and December, 1944, the differences were, however, significant. In the black-quarter area they were 11·4, 18·1 and 15·1 per 1,000 head of population for each of the three months, while in the non-black-quarter area the mortality remained at the expected dry-season level of five per 1,000 or less. The high losses in the black-quarter region are also significant when the mortality during 1943 is compared with that of 1944.

Experience gained during the three years of the survey showed that, in the absence of any epizootics, the expected disease mortality during October to December would not exceed 5 per 1,000 head of

population. During October, November and December, 1944, 6·4, 13·1 and 10·1 beasts respectively died in excess of this expected five beasts per 1,000 head. In a population of 36,000 head, this excess represents 230 death in October, 472 in November and 364 in December, or a total loss of 1,066 head. The actual total mortality figures for each tank area during the epizootic are given in Table IV, while the losses per 1,000 head of population is shown in Fig. 2.

TABLE IV

MORTALITY FROM DISEASE IN EACH TANK AREA DURING PERIOD OF BLACK-QUARTER EPIZOOTIC

| *Name of tank* | *Cattle population 1st August*, 1944 | *Mortality from all diseases* 1944 | | | | |
|---|---|---|---|---|---|---|
| | | *Aug.* | *Sept.* | *Oct.* | *Nov.* | *Dec.* |
| Likuni ... | 3,305 | 24 | 13 | 40 | 33 | 24 |
| Chadza ... | 3,123 | 8 | 10 | 45 | 30 | 60 |
| Jumpha ... | 3,547 | 20 | 15 | 55 | 119 | 74 |
| Lincadzi ... | 2,111 | 18 | 8 | 9 | 7 | 20 |
| Namitete ... | 2,736 | 13 | 8 | 10 | 14 | 18 |
| Chilinda ... | 2,639 | 12 | 11 | 35 | 13 | 25 |
| Mbabzi ... | 4,929 | 49 | 30 | 51 | 88 | 54 |
| Malandi ... | 4,674 | 13 | 3 | 33 | 79 | 78 |
| Lombadzi ... | 4,298 | 13 | 20 | 99 | 164 | 63 |
| Magiga ... | 3,685 | 13 | 19 | 36 | 116 | 134 |
| *Totals* ... | 35,047 | 183 | 137 | 413 | 663 | 550 |

During the dry months of August and September, just before the epizootic started, the average mortality rate was 160 beasts per month. During the three months of October to December, 253, 503 and 390 beasts died in excess of this average or a total of 1,146 head. The very numerous field observations and reports received all showed that this increase was due to black-quarter. It can, therefore, be assumed with reasonable accuracy that between 1,000 and 1,200 head of cattle died of the disease in this area, representing a mortality rate of 28 to 33 per 1,000 head of population for the three month period.

### VARYING MORTALITY IN DIFFERENT AREAS

While the average mortality rate over the whole area was nine to ten beasts per 1,000 each month of the epizootic, the losses in several areas was very significantly in excess of this figure. The figures for Lombadzi and Magiga, two adjacent tank areas, are given in Table V. In Lombadzi the black-quarter outbreak occurred in October, while in Magiga no definite cases occurred until November. In Magiga area there was, however, a slight unaccountable rise in mortality during May and June, 1944.

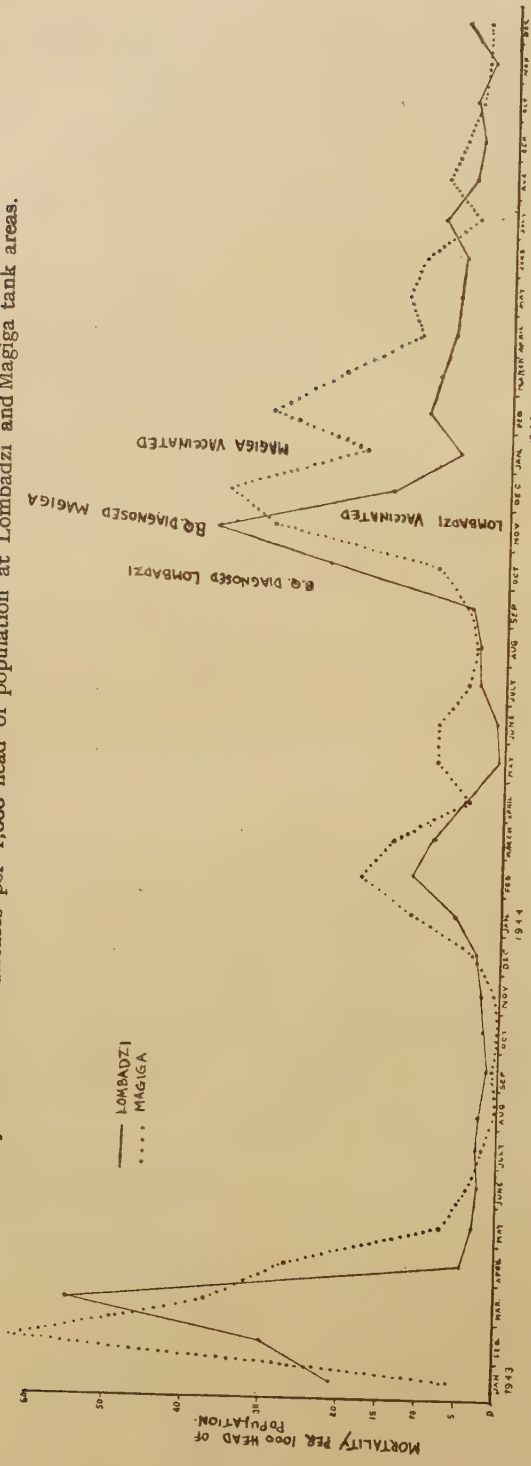



FIG. II

Mortality rate for all diseases per 1,000 head of population at Lombadzi and Magiga tank areas.

TABLE V

MORTALITY FROM DISEASE AT LOMBADZI AND MAGIGA TANK AREAS DURING 1943–45

| | *Lombadzi* | | | *Magiga* | | |
|---|---|---|---|---|---|---|
| | *Cattle population 1st of each month* | *Total deaths from disease* | *Deaths from disease per 1,000* | *Cattle population 1st of each month* | *Total deaths from disease* | *Deaths from disease per 1,000* |
| 1943 | | | | | | |
| Jan. | 5,367 | 126 | 21·0 | 4,454 | 38 | 8·0 |
| Feb. | 5,108 | 152 | 30·0 | 4,418 | 275 | 62·0 |
| Mar. | 4,805 | 266 | 55·0 | 3,862 | 133 | 37·0 |
| Apr. | 4,525 | 20 | 4·4 | 3,713 | 103 | 27·0 |
| May | 4,515 | 14 | 3·1 | 3,625 | 28 | 7·0 |
| June | 4,513 | 11 | 2·4 | 3,633 | 15 | 4·0 |
| July | 4,508 | 13 | 2·9 | 3,630 | 9 | 2·0 |
| Aug. | 4,490 | 11 | 2·5 | 3,642 | 1 | 0·2 |
| Sept. | 4,518 | 8 | 1·8 | 3,650 | 3 | 0·8 |
| Oct. | 4,540 | 9 | 2·0 | 3,650 | Nil | Nil |
| Nov. | 4,623 | 11 | 2·4 | 3,789 | 3 | 0·8 |
| Dec. | 4,614 | 14 | 3·2 | 3,806 | 14 | 3·6 |
| 1944 | | | | | | |
| Jan. | 4,588 | 27 | 6·0 | 3,762 | 44 | 11·7 |
| Feb. | 4,518 | 52 | 11·5 | 3,678 | 70 | 18·0 |
| Mar. | 4,459 | 38 | 9·0 | 3,623 | 53 | 14·0 |
| Apr. | 4,308 | 22 | 5·1 | 3,521 | 17 | 4·8 |
| May | 4,243 | 4 | 1·0 | 3,500 | 30 | 8·5 |
| June | 4,289 | 5 | 1·0 | 3,530 | 30 | 8·5 |
| July | 4,301 | 15 | 3·5 | 3,580 | 18 | 5·0 |
| Aug. | 4,298 | 13 | 3·5 | 3,685 | 13 | 3·8 |
| Sept. | 4,392 | 20 | 4·6 | 3,799 | 19 | 5·0 |
| Oct. | 4,394 | 99 | 22·0 | 3,816 | 36 | 9·0 |
| Nov. | 4,367 | 164 | 37·5 | 3,777 | 116 | 30·0 |
| Dec. | 4,261 | 63 | 15·0 | 3,684 | 134 | 36·0 |
| 1945 | | | | | | |
| Jan. | 4,178 | 27 | 6·5 | 3,576 | 66 | 18·5 |
| Feb. | 4,199 | 45 | 10·7 | 3,520 | 107 | 30·4 |
| Mar. | 4,158 | 39 | 9·4 | 3,381 | 72 | 21·3 |
| Apr. | 4,138 | 32 | 7·7 | 3,300 | 39 | 11·8 |
| May | 4,142 | 29 | 7·0 | 3,294 | 45 | 13·7 |
| June | 4,176 | 26 | 6·2 | 3,253 | 37 | 11·4 |
| July | 4,197 | 40 | 9·5 | 3,259 | 15 | 4·6 |
| Aug. | 4,276 | 22 | 5·1 | 3,349 | 30 | 8·9 |
| Sept. | 4,332 | 18 | 4·2 | 3,414 | 22 | 6·4 |
| Oct. | 4,397 | 23 | 5·2 | 3,455 | 15 | 4·3 |
| Nov. | 4,376 | 13 | 2·9 | 3,508 | 13 | 3·7 |
| Dec. | 4,397 | 28 | 6·4 | 3,497 | 13 | 3·7 |

For comparison, the mortality rates in two areas where black-quarter appeared to be enzootic are given in Table VI. In the Mbabzi area, deaths above the expected five per 1,000 occurred during the dry months of June, July and again in September, 1943. Throughout the dry season of 1944 the rate in this area remained consistently above five per 1,000, but it was only in October, when the author was fully aware of the importance of *Cl. chauvoei* infection that the

disease was definitely diagnosed. The highest dry season mortality rate in this area was 17·4 per 1,000 in November compared with 37·5 per 1,000 in Lombadzi area.

TABLE VI

MORTALITY FROM DISEASE AT MBABZI AND CHILINDA TANK AREAS DURING 1943–1945

| | *Mbabzi* | | | *Chilinda* | | |
|---|---|---|---|---|---|---|
| | *Cattle population 1st of each month* | *Total deaths from disease* | *Death rate per 1,000* | *Cattle population 1st of each month* | *Total deaths from disease* | *Death rate per 1,000* |
| 1943 | | | | | | |
| Jan. | 6,319 | 36 | 5·7 | | No records | |
| Feb. | 6,233 | 54 | 8·8 | | | |
| Mar | 6,081 | 112 | 18·4 | 2,807 | 50 | 17·8 |
| Apr. | 5,810 | 41 | 7·1 | 2,112 | 39 | 18·4 |
| May | 5,815 | 31 | 5·3 | 2,610 | 29 | 11·1 |
| June | 5,854 | 90 | 15·3 | 2,592 | 23 | 8·8 |
| July | 5,890 | 47 | 7·9 | 2,614 | 20 | 7·6 |
| Aug. | 5,926 | 28 | 4·7 | 2,610 | 8 | 3·0 |
| Sept. | 5,948 | 66 | 11·1 | 2,624 | 9 | 3·4 |
| Oct. | 5,999 | 34 | 5·9 | 2,855 | 4 | 1·4 |
| Nov. | 6,226 | 27 | 4·3 | 2,863 | 3 | 1·0 |
| Dec. | 6,210 | 32 | 5·1 | 2,886 | 5 | 1·7 |
| 1944 | | | | | | |
| Jan. | 6,150 | 77 | 12·5 | 2,879 | 16 | 5·5 |
| Feb. | 6,019 | 95 | 15·6 | 2,837 | 36 | 12·6 |
| Mar. | 5,905 | 144 | 24·4 | 2,740 | 32 | 11·6 |
| Apr. | 4,830 | 50 | 12·4 | 2,678 | 53 | 19·7 |
| May | 4,795 | 39 | 8·1 | 2,599 | 29 | 11·1 |
| June | 4,839 | 34 | 7·0 | 2,595 | 17 | 6·5 |
| July | 4,889 | 44 | 9·0 | 2,596 | 26 | 10·0 |
| Aug. | 4,929 | 49 | 10·0 | 2,639 | 12 | 4·5 |
| Sept. | 5,030 | 30 | 5·9 | 2,759 | 11 | 3·9 |
| Oct. | 5,073 | 51 | 10·0 | 2,804 | 35 | 12·4 |
| Nov. | 5,070 | 88 | 17·4 | 2,864 | 13 | 4·5 |
| Dec. | 5,112 | 54 | 10·5 | 2,870 | 25 | 8·7 |
| 1945 | | | | | | |
| Jan. | 5,140 | 97 | 18·9 | 2,818 | 45 | 15·9 |
| Feb. | 4,954 | 91 | 18·4 | 2,768 | 85 | 30·7 |
| Mar. | 4,885 | 82 | 16·8 | 2,672 | 59 | 22·1 |
| Apr. | 4,804 | 91 | 18·9 | 2,587 | 53 | 20·5 |
| May | 4,746 | 60 | 12·6 | 2,510 | 34 | 13·5 |
| June | 4,734 | 55 | 11·6 | 2,498 | 21 | 8·4 |
| July | 4,763 | 55 | 11·5 | 2,497 | 21 | 8·4 |
| Aug. | 4,805 | 60 | 12·5 | 2,521 | 12 | 4·8 |
| Sept. | 4,921 | 40 | 8·1 | 2,844 | 2 | 0·7 |
| Oct. | 4,964 | 20 | 4·0 | 2,568 | 4 | 1·6 |
| Nov. | 5,059 | 52 | 10·2 | 2,588 | 11 | 4·2 |
| Dec. | 5,091 | 37 | 7·3 | 2,616 | 12 | 4·6 |

The history at Chilinda tank area is not quite so clear, but here again, although the disease was not definitely diagnosed until October, investigation did show that it had occurred previously, especially during July, 1944, when mortality was 10·0 per 1,000.

Thus, within the relatively small area investigated we had two differing pictures for the mortality rate. In regions such as Lombadzi and Magiga, there was no definite evidence that the disease had occurred within at least the last five years. When the epizootic arose in October, 1944, the mortality rate was high, reaching 37·5 per 1,000 in November. In other parts exemplified by Mbabzi and Chilinda, undiagnosed deaths have undoubtedly occurred from black-quarter over many years and so no very definite epizootic wave occurred in October-December, 1944, when the disease was prevalent. The low figures for Likuni area were due to the fact that the disease was diagnosed early and all susceptible cattle were promptly vaccinated. But probably the most important factor restricting mortality was the localisation of the epizootic to relatively few herds in the area, *i.e.*, the disease could be associated with definite pastures.

### AGE INCIDENCE OF DISEASE

Mortality, as might be expected, occurred chiefly amongst immature animals. Deaths were recorded under three classes, adults, immature cattle one to three years old and calves one year old or under. The mortality in Jumpha tank for 1944-45 under these classes is given in Table VII. Age, however, can be given only for those cases from which spleen smears were received.

There was a significant increase in deaths in cattle one to three years old and in calves one year old and under during October to December, 1944, when black-quarter was epizootic. The total mortality rate per 1,000 head of cattle in Jumpha area is shown in Fig. 3.

FIG. III

Disease mortality rate per 1,000 of population in Jumpha Tank area during 1944.

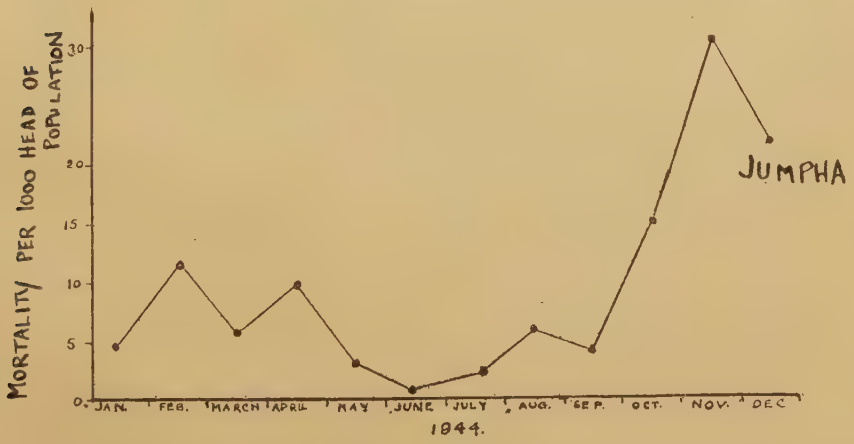


TABLE VII

MORTALITY FROM EACH AGE GROUP IN JUMPHA TANK AREA DURING 1944–45

| | *Cattle population 1st of each month* | *Mortality according to age group* | | | *Total* | *Total unsmeared* | *Total deaths from disease* | *Disease mortality per 1,000 of population* |
|---|---|---|---|---|---|---|---|---|
| | | *Adults* | *1–3 years old* | *1 year or under* | | | | |
| 1944 | | | | | | | | |
| Jan. | 4,282 | 4 | 8 | 3 | 15 | 4 | 19 | 4·4 |
| Feb. | 4,246 | 6 | 6 | 11 | 23 | 26 | 49 | 11·5 |
| Mar. | 4,213 | 6 | 3 | 13 | 22 | 1 | 23 | 5·5 |
| Apr.* | 3,379 | 4 | 6 | 10 | 20 | 13 | 33 | 9·7 |
| May | 3,351 | 2 | 2 | 6 | 10 | 0 | 10 | 3·0 |
| June | 3,405 | 12 | 3 | 3 | 18 | 5 | 23 | 7·0 |
| July | 3,523 | 0 | 4 | 4 | 8 | 1 | 9 | 2·1 |
| Aug. | 3,547 | 2 | 6 | 8 | 16 | 4 | 20 | 6·3 |
| Sept. | 3,629 | 4 | 6 | 5 | 15 | 0 | 15 | 4·2 |
| Oct. | 3,649 | 8 | 22 | 21 | 51 | 4 | 55 | 15·1 |
| Nov. | 3,575 | 17 | 41 | 60 | 118 | 1 | 119 | 30·5 |
| Dec. | 3,430 | 12 | 22 | 39 | 73 | 1 | 74 | 21·5 |

* 700 head approximately transferred to Lingadzi tank.

RESULTS OF VACCINATION DURING OUTBREAK

Black-quarter was first definitely diagnosed in 1944, and it was difficult, therefore, to plan an immunisation programme as the severity of the infection and the exact geographic limits of the infected area were still unknown. In this initial outbreak, therefore, the policy in each region was to wait until the disease was definitely diagnosed before deciding on preventive measures. The doubtful value of vaccination applied during the time of the actual outbreak was realised as it would take at least ten days for immunity to develop. During that time cattle of susceptible age might be rendered more susceptible to infection and vaccination might, therefore, increase mortality. In addition, the transport charges of the vaccine from Onderstepoort, Pretoria, were heavy, so that the cost of the vaccination campaign had to be considered. Further, it was reasonable to assume that the definite waves of mortality would be succeeded by periods of quiescence and that, with the onset of the rains in December and the change of grazing grounds that this would necessitate, the epizootic would cease of its own accord. Such a sequence of events was actually seen at Mbabzi area.

These considerations could not, however, be easily explained to native cattle-owners who were losing stock rapidly and expected the veterinary officer to produce a remedy. The African veterinary assistants in the affected tank areas also anticipated some action for reasons of prestige. There was also the possibility that early vaccination might cause some lessening of the severity of the epizootic and in any case if the mortality did decrease after vaccination the result would be attributed by the cattle owners to the result of vaccination. Further, if a vaccination campaign was undertaken,

considerable useful experience would be gained by the African staff in mass vaccination procedure and the immunity conferred on the vaccinated stock would protect them for some portion of the dry season in 1945. Lastly, vaccination was the only possible remedy as infected carcases were cut up and eaten by Africans and grazing grounds near villages heavily contaminated by infective bacilli in spite of all veterinary police measures. A vaccination programme for each infected area was, therefore, decided on with as little delay as possible. An alum-precipitated vaccine of *Cl. chauvoei* was obtained from Onterstepoort Veterinary Laboratories; *Cl. septique* was not incorporated as it was considered that this bacterium was not heavily involved as a cause of mortality in this outbreak. Seven tank areas were vaccinated, and Table VIII shows the numbers of cattle vaccinated in each area

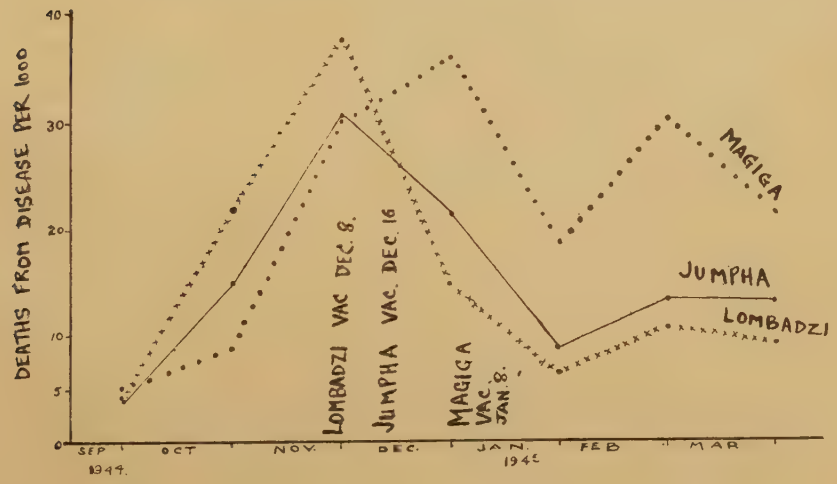
TABLE VIII

NUMBER OF CATTLE, 9 MONTHS TO 3 YEARS OLD, VACCINATED IN EACH OF SEVEN TANK AREAS

| *Name of tank Area* | *Nos. vaccinated* | *Date of vaccination* |
|---|---|---|
| Likuni ... ... | 1,687 | 27.11.44–2.12.44 |
| Jumpha ... ... | 1,608 | 19.12.44–21.12.44 |
| Malandi ... ... | 2,249 | 19.12.44–24.12.44 |
| Chikuse ... ... | 245 | 17.1.45 |
| Lombadzi .... | 1,594 | 8.12.44–11.12.44 |
| Magiga ... ... | 1,619 | 5.12.44– 9.12.44 |
| Chadza ... ... | 1,123 | 25.12.44–30.12.44 |
| | 9,525 | |

FIG. IV

Mortality rates in the tank areas following black-quarter vaccination.

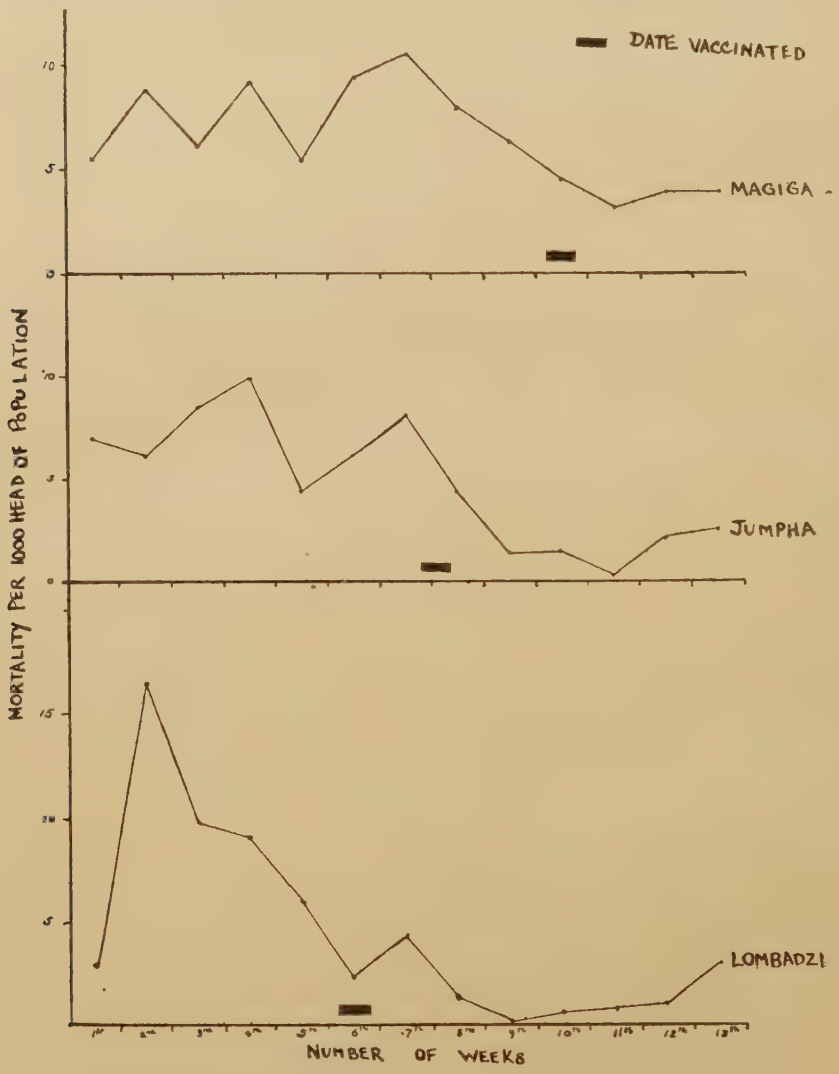


The effect of vaccination on the mortality curve of three tank areas is shown in Fig. 4. The date of vaccination recorded on this figure is that on which most of the cattle were vaccinated.

A striking fall in the mortality rate in all three areas following vaccination is apparent. During November the losses in the Lombadzi area were 37·5 per 1,000, but following vaccination on, or about, December 8th, in December they fell to 15·0 per 1,000 (Table V). In Magiga area the rate of 36·0 head in December fell

FIG. V

Mortality from disease during a 13-week period from November 1st, 1944 to January 30th, 1945 in three tank areas.



after vaccination about January 8th to 18·5 in January. In Jumpha area the rate of 30·5 head in November fell to 21·5 after vaccination of stock about December 19th. The question arises, however, as to how much of this fall in mortality was due to a natural decline in the epizootic, and how much was due to vaccination. To answer this question, the mortality curve for each week of the epizootic is given in Table IX and shown in Fig. 5.

TABLE IX

WEEKLY DISEASE MORTALITY RATE IN MAGIGA, JUMPHA AND LOMBADZI AREAS FROM NOVEMBER 1ST, 1944 TO JANUARY 30th, 1945

| *No. of Week* | *Date* | *Magiga* | | *Jumpha* | | *Lombadzi* | |
|---|---|---|---|---|---|---|---|
| | | *Total deaths from disease* | *Deaths per 1,000 of population* | *Total deaths from disease* | *Deaths per 1,000 of population* | *Total deaths from disease* | *Deaths per 1,000 of population* |
| 1 | 1.11.44–7.11.44 | 21 | 5·5 | 28 | 7·9 | 13 | 2·9 |
| 2 | 8.11.44–14.11.44 | 29 | 7·7 | 22 | 6·1 | 72 | 16·5 |
| 3 | 15.11.44–21.11.44 | 23 | 6·1 | 30 | 8·4 | 43 | 9·8 |
| 4 | 22.11.44–28.11.44 | 35 | 9·2 | 35 | 9·8 | 40 | 9·1 |
| 5 | 29.11.44–5.12.44 | 20 | 5·4 | 15 | 4·4 | 25 | 5·9 |
| 6 | 6.12.44–12.12.44 | 31 | 8·4 | 21 | 6·1 | 10 | 2·3 |
| 7 | 13.12.44–19.12.44 | 40 | 10·8 | 28 | 8·1 | 19 | 4·4 |
| 8 | 20.12.44–26.12.44 | 28 | 7·8 | 15 | 4·4 | 6 | 1·4 |
| 9 | 27.12.44–2.1.45 | 23 | 6·2 | 5 | 1·4 | 1 | 0·2 |
| 10 | 3.1.45–9.1.45 | 16 | 4·5 | 5 | 1·5 | 3 | 0·7 |
| 11 | 10.1.45–16.1.45 | 11 | 3·1 | 1 | 0·3 | 4 | 0·9 |
| 12 | 17.1.45–23.1.45 | 14 | 3·9 | 7 | 2·1 | 5 | 1·2 |
| 13 | 24.1.45–30.1.45 | 14 | 3·9 | 8 | 2·5 | 13 | 3·1 |

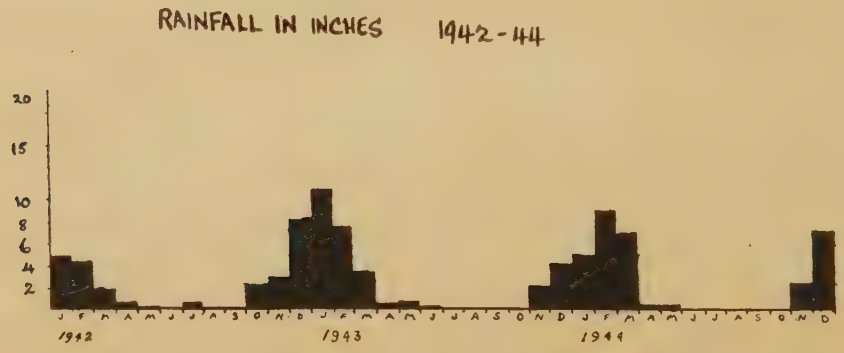
It will be seen that the rate at Magiga tank area was actually decreasing *before* vaccination and owing to delay in vaccination, the course of the epizootic was probably unaltered. In Jumpha tank area the rate was also declining but vaccination probably prevented the occurrence of fresh mortality waves, such as had occurred on the 4th and 7th week of the epizootic. In the Lombadzi area a small wave did occur the week following vaccination, but the increased mortality rate was small and there were no further waves. Nevertheless, vaccination during an epizootic is of very limited value, and the results are far inferior to those obtained when it is used as a preventive some months prior to expected outbreaks.

## DISCUSSION

For the first time in Nyasaland accurate statistics are given for mortality in cattle due to disease before, during and after an epizootic of black-quarter. From a study of these figures, there is every reason to believe that black-quarter is a dry season disease. The rise in mortality which occurs regularly during the rains is most probably due to tick-borne protozoan infections and bears no relation to *Cl. chauvoei* infections as this mortality occurs in areas where

black-quarter is known to be absent; moreover, vaccination of herds in Likuni region did not prevent this wet season rise in mortality. In a recent paper (Wilson, 1946) the vegetation of the Lilongwe area is briefly described. During the rainy months of December to April cattle chiefly graze in the dry *Brachystegia*—open grassland areas—and are largely excluded from the more fertile cultivated areas dominated by *Combretun-Acacia* associations, and are mostly entirely excluded from the seasonally saturated "dambo" marsh lands. Grazing is also more luxurious during this growing season, and herd boys have a greater choice of watering places. During the dry season cattle have theoretically a greater choice since they can graze unrestricted on the cultivated areas when crops have been reaped and also on the rapidly drying dambo areas. In actual fact, grazing is more restricted as most of the tall grass becomes dry and woody and is eventually burned, while the areas which carry more palatable grasses, *e.g.*, villages carrying *Cynodon* species and the greener grasses of the damboes, are closely grazed. Evidence is often found *post-mortem* of soil being ingested. Naturally, the general state of the grazing during September, October and November depends largely on the amount of rainfall of the previous rainy season. The rainfall over a three-year period is shown in Fig. 6.

FIG. VI




The dry season in 1942 was broken by slight rainfall in July and relatively heavy rainfall in October and November. The rains in 1943 were prolonged until June, and the total rainfall from October, 1942, until March, 1943, was 35·4 inches or two inches above the average of 33·4 inches over the last 13 years. No real shortage of grazing occurred during either of these dry seasons, and no black-quarter was diagnosed. During the rainy period from October, 1943, to March, 1944, only 27·83 inches fell. This was badly distributed and followed by a very hot dry season, the highest daily recordings during this three-year period (1942-44) being on October 31st to November 3rd, 1944, when maximum daily temperatures

rose above 96° F. each day. Grazing was generally poor and restricted and it seems reasonable to correlate this fact with the abnormally high mortality from *Cl. chauvoei*. In areas like Mbabzi tank area, where over-stocking and considerable soil erosion normally occurs, it appears certain that small minor outbreaks of black-quarter constantly occur throughout the dry season due probably to close overgrazing of infected pastures.

The economic loss to cattle owners following the death of over 1,000 young beasts due to *Cl. chauvoei* must represent a sum of approximately £2,000 per annum, whereas the total cost of vaccination during 1944 was about £200. That vaccination in some cases proved useless was largely due to the fact that the outbreak occurred without any previous warning. Now that the infected areas have been demarcated a more rational vaccination policy will be possible in future years.

## SUMMARY

Black-quarter was definitely diagnosed in the Central Province of Nyasaland for the first time during October to December, 1944. A map is given to show the restricted area affected.

The general mortality in cattle showed an increased incidence of over ten head per 1,000 of the cattle population during each of the wet hot months of December to April and a low mortality of under five per 1,000 during each of the dry months of May to November. The black-quarter epizootic upset this seasonal incidence of disease and an increased mortality of 37 per 1,000 occurred during October and November.

It is estimated that between 1,000 and 1,200 head of young cattle died as a result of the black-quarter epizootic.

Two types of wave occurred in the area. A high mortality was noted in areas where the disease was truly epizootic, while mortality was lower in areas where the disease was more enzootic. The greatest losses occurred in cattle of immature age. Vaccination was generally undertaken too late to be fully effective.

Evidence suggests that in Nyasaland, black-quarter is a disease of the dry season.

## REFERENCES

Hornby, H. E. (1933). *Annual Rep. Dept. vet. Sc. anim. Hus., 1932.* Gov. Printer; Dar es Salaam.

Smith, J. (1931). *Annual Rep. Dept. anim. Health, 1930.* Gov. Printer; Lusaka.

Wilson, S. G. (1936). *Parasitol.* (in press).

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# IMMUNOLOGICAL PHENOMENA IN THE CALF, FOLLOWING EXPERIMENTAL INFECTION WITH *BUNOSTOMUM PHLEBOTOMUM*

By

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## INTRODUCTION

Although it has been shown that skin penetration is the normal mode of infection for *Bunostomum phlebotomum* (Mayhew, 1939, Sprent, 1946) it has been observed by the present writer that the periodic application of larvae to the skin of calves is not a satisfactory method for inducing a heavy infestation.

Mayhew (1940) noted that the periodic application of a mixture of Bunostomum and Oesophagostomum larvae to the skin of the calf caused a gradual rise in the egg output in the faeces until a peak was reached, but the rise was not proportional to the number of larvae applied. The peak was followed by a sudden decrease, sometimes accompanied by diarrhoea, after which the egg counts remained at a very low level, in spite of repeated administrations of larvae. These observations suggest that the calf, as a result of a series of infections, acquires some mechanism whereby it can either prevent the entry of larvae into its body or destroy them within the body or prevent the adults from laying eggs. Abundant evidence has now accumulated to show that mammals infected with helminth parasites acquire a defence mechanism which enables them to control the extent of their parasite burden. This mechanism has been found to be especially active in the case of infestation with parasites which come into intimate contact with the somatic tissues of the host. Intestinal nematodes, the infective larvae of which gain access to the host by penetration of the skin, are therefore particularly liable to be influenced by such a mechanism, as has been established in the case of *Nippostrongylus muris* in the rat (Taliaferro and Sarles, 1939), *Ancylostoma caninum* in the dog (Otto and Kerr, 1939), and *Strongyloides ratti* in the rat (Sheldon, 1937). The reaction appears to be due partly to humoral factors, whereby antigenic substances in and around the larvae and adult parasites are precipitated in such a way as to inhibit the progress or development of the worms in the body of the host, and partly to cellular factors whereby the incapacitated larvae are engulfed and destroyed or encapsulated. The skin, lungs and intestine appear to be the main sites of interaction between host and parasite (Taliaferro and Sarles, 1939).

Now that it has been established that the infective larvae of *Bunostomum phlebotomum* enter the calf through the skin and migrate through the lungs on their way to the duodenum (Sprent, 1946), it is to be expected that the calf will acquire an active resistance against this parasite. The present paper records the results of a few experiments, carried out in Nigeria, designed to show whether or not some such mechanism exists. The calves were subjected to a series of cutaneous infections and observations made on (1) the local reaction in the skin to the penetration of larvae; (2) the sensitivity of the skin to extracts of the adult parasite; (3) the effect of extracts of

the adult parasite in stimulating serum antibodies; (4) the resistance of the calf to natural reinfection at pasture. Some observations on the cellular reaction to the adult parasite in the duodenum are also recorded.

## METHODS

*Experimental animals.* Six Zebu calves (A—F) three to six months old, were obtained from a farm where they had been kept in pens and pail-fed from birth. At the start of the experiment no helminth eggs were detected in the faeces, except those of *Strongyloides papillosus* in Calf E. Throughout the experiment the calves were kept in separate pens and cared for by a special attendant. Their food consisted of milk, guinea-corn bran and hay, the last being collected from the writer's own garden, where no bovine hookworm eggs were likely to occur.

*Cultures.* Infective larvae were obtained from cultures of uterine eggs mixed with sterile ruminal contents and collected by the Baermann technique.

*Application of larvae to the skin.* The larvae were applied in drops of water to a small area 8 × 15 mm. on the neck which was shaved, rinsed and demarcated with a grease pencil. When the larvae had sunk in the drop to the surface of the skin, the fluid was carefully removed by blotting paper so as to make room for another drop. The process was repeated until all the fluid containing the larvae was applied. The animal was cast on its side while the larvae were being applied and the site of application was watched and guarded for 30 minutes afterwards. At the end of this time washings from the skin surface were examined for larvae to make sure that satisfactory penetration had occurred.

*Extracts of B. phlebotomum.* The extracts were made by cutting the adult parasites into very small pieces and drying them in a Cryochem apparatus. The dried residue was ground to a fine powder, weighed and dissolved in 100 times its weight of Coca's fluid. A little chloroform or toluol was added to prevent putrefaction and the mixture was left at 37° C. for three days. It was then centrifuged by hand and the slightly opalescent supernatant fluid was used as the "extract."

*Histological technique.* After the desired period had elapsed following the application of larvae to the skin, the area of penetration was excised and dropped into Helly's fixative. The wound was immediately sprayed with cocaine and adrenaline, and then sutured. After fixation and washing, the piece of skin was softened in 5 per cent. phenol for three days.

To obtain sections of the adult parasite *in situ*, the duodenum was opened soon after death and Helly's fixative at 60° C. poured over the worms. A small piece of tissue with the parasite attached was cut out with scissors and a single strand of cotton tied round it

to keep the parasite in its original position throughout the subsequent manipulations.

Skin and duodenum were dehydrated in dioxan, embedded in paraffin wax, and stained with dilute Haematoxylin, followed by Eosin and Azure II. This method gives a very satisfactory picture of the adult hookworm in the duodenum because the cuticle of the buccal capsule, dorsal cone, buccal lancets and oesophagus is stained a deep blue, while the cuticle of its body wall is stained pink.

*Precipitin test.* Undiluted extract was used as antigen in a simple ring test. The serum was overlaid by the extract, and the tubes, with appropriate controls, were incubated for 30 minutes at 37° C. A well defined white ring at the junction of the two fluids was regarded as a positive test.

*Intradermal test.* The extract was diluted 10 times with 0·9 per cent. sodium chloride solution and 0·1 c.c. was injected intradermally into the caudal fold or into a shaved area of the neck. 0·1 c.c. of Coca's fluid similarly diluted was injected into the other fold or, in the case of cervical injections, about four inches away from the extract. The swollen area was measured by a ruler and by calipers after 15 minutes. If the diameter or the thickness of the swollen area after 15 minutes was three times the diameter or thickness immediately after injection it was regarded as a positive result.

*Experimental procedure.*

Calf A and Calf B were infected twice weekly for two months by the application to the skin of different parts of the body of about 2 c.c. of a thick suspension of larvae in water.

Calf C was infected similarly every other day.

Calf D was injected twice weekly with extract of *B. phlebotomum* via the jugular vein. Sixteen injections were given, the first nine consisting of regularly increasing doses (1 c.c. to 9 c.c.) of extract, each of which was made up to a volume of 10 c.c. by the addition of sterile 0·9 per cent. sodium chloride solution: the seven subsequent injections each contained 10 c.c. of undiluted extract.

Calves E and F were kept as controls.

## OBSERVATIONS AND RESULTS

### *Cutaneous reactions*

It was noticed that whenever calves were subjected to a series of infections by the application of larvae to the skin, both the rate of development and the extent of the local reaction increased with each successive exposure. Some observations on Calf C, which was infected on alternate days, may serve as an example. At the first infection there was a slight swelling 60 minutes after application; at 24 hours the area of penetration showed small isolated scabs and there was very little increase in the lesion up to five days later. At the fourth infection there was slight swelling at 60 minutes, at 24 hours the area was rough and scabby, and at four days the lesion

had increased to a maximum extent of about one inch. At the eighth infection there was slight swelling at 60 minutes and at 24 hours there was a thick coalescing scab, which extended into a large circular scabby plaque about four inches in diameter. At the ninth infection there was marked swelling at 60 minutes and pain was evidently felt by the calf since it attempted to lick the site. At 24 hours the area was an open sore which developed into a large plaque covered by a rough scab. After reaching a maximum intensity the reactions to further successive infections then began to subside until very little change was evident following the application of larvae.

It has been shown (Sprent, 1946) that the larvae reach the deeper layers of the dermis within 30 minutes of application to the surface of the skin and that they remain in the skin for a few hours only. It seems, therefore, that the speed of onset of the local defence reaction, if such exists, is of prime importance if it is to be effective, and that if the reaction does not begin within an hour of application of the larvae it may well be ineffective in checking their entry. If the macroscopic changes visible on the area of penetration are indicative of a defence reaction it appears that only as a result of several previous infections, is the calf able to react quickly enough to attack the larvae in the skin. Some information on the nature of the mechanism was obtained by comparing sections of the skin of calves A and E removed 60 minutes after application of larvae. Calf A had previously been subjected to eight infections, administered twice a week, and calf E had had no previous infections. Three days after the eight infection of Calf A, 200 larvae were applied to the skin of each calf and the area of penetration was watched for 60 minutes and then cut out and dropped into fixative.

Macroscopic changes: In Calf A, 15 minutes after application of the larvae the site was swollen and the calf evidently felt pain, as it kicked and attempted to lick the site. After 20 minutes an area 25 mm. in diameter was swollen. In Calf E, on the other hand, no change was evident at 15 minutes and even at 60 minutes there was only slight swelling and no sign of pain or irritation.

Microscopic changes: The sections of skin removed after 60 minutes revealed that the blood vessels in the dermis of Calf A were packed with neutrophil leucocytes, many of which were migrating into the surrounding tissues, while very few leucocytes were evident in the dermis of Calf E (see Figs. 1 and 2).

These observations suggest that after several reinfections the calf acquires the ability to react to the penetration of larvae by vascular and cellular changes which increase in speed and intensity.

To investigate whether such a reaction indicates a developing resistance to infection, *i.e.*, whether the calf is able to prevent the larvae from getting through the skin by means of an acquired mechanism, the area of penetration in the neck of Calf C was

FIG. 1.

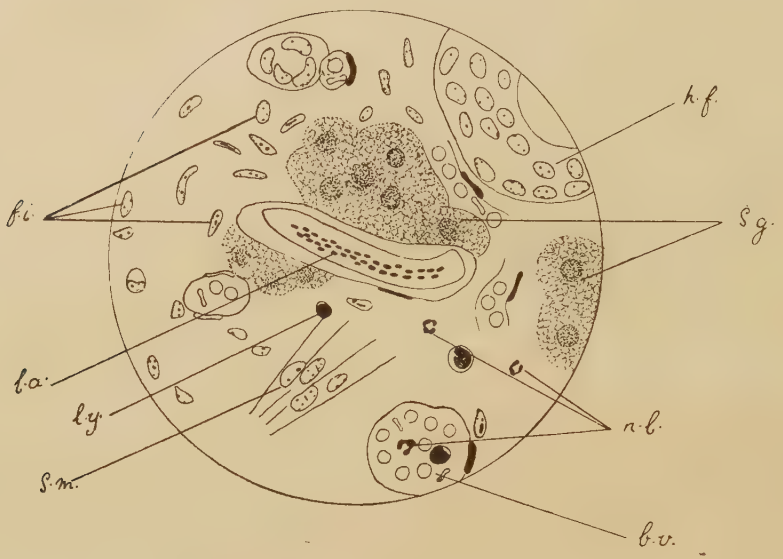


FIG. 2.

FIG. 1.—Infective larva lying in the dermis, 60 minutes after application to the skin. First infection. ×400.

FIG. 2.—Infective larva lying in the dermis, 60 minutes after application to the skin. Eighth infection. ×400.

LEGEND.

| | | | | | |
|---|---|---|---|---|---|
| b.v. | = | blood vessel. | ly. | = | lymphocytes. |
| fi. | = | fibroblasts. | n.l. | = | neutrophil leucocytes. |
| h.f. | = | hair follicle. | s.g. | = | sebaceous glands. |
| la. | = | larva. | s.m. | = | smooth muscles. |

removed 18 hours after the first, the eighth and the thirteenth infection. The precise number of larvae applied on each occasion is not known, but they were approximately equal. A piece of normal skin was also removed from this calf before exposure to infection, using exactly the same technique of washing, shaving and excising the skin.

The normal skin showed no damage to the epidermis and the stratum corneum was intact along the whole length of the sections. Between the stratum corneum and the epidermal cells, a few small clusters of neutrophil leucocytes were seen. In the dermis there was a variety of mesenchymal haematogenous and histogenous cellular elements. Typical small lymphocytes were present, mostly collected around the blood vessels, together with a variety of larger cells with more lightly staining nuclei than the small variety, but still spherical or oval in shape, and with increased cytoplasm which had lost its basophilia to a greater or lesser extent. Of the histogenous cellular elements there were fibroblasts and smooth muscle cells, very occasional large histogenous macrophages with lobular nuclei and grey vacuolated cytoplasm, and a considerable number of cells with basophil granules in the cytoplasm. The last named showed considerable variation between cells with a round or oval nucleus and very small dustlike basophil cytoplasmic granules, and cells with an elongated, very deeply staining nucleus and with the cytoplasm packed with larger deeply staining granules.

None of the sections of skin removed after the first, the eighth and the thirteenth applications of larvae contained larvae, which had all apparently escaped into the blood stream. There was evidence of very considerable damage to the epithelium, probably caused by the larvae. Sections showed that the larvae, having penetrated through the epidermis, travel towards the hair-follicles by burrowing between the epidermal cells and the dermis. Having reached a hair-follicle they appear to descend mostly into the sebaceous glands and reach the dermis by breaking down the sebaceous cells. During the progress of the larvae considerable numbers of epidermal cells must be loosed from their attachment. In some parts of the sections the epithelial cells were obliterated by masses of neutrophil and eosinophil leucocytes (Fig. 3), which could be observed migrating between the epithelial cells from the neighbouring blood vessels. The dermis showed no gross changes from the normal skin except for an increase in the number of neutrophil and eosinphil leucocytes. There was a slight increase in the cells with basophil granules, and the lymphoid cells appeared to have been more active at the time of fixation, their cytoplasm being extended into pseudopodia-like processes. In some of them the nuclei had become horseshoe-shaped and they were evidently undergoing changes into the polyblast form. No marked differences were to be noted between the sections removed after the first, the eighth and the thirteenth infections.

In summarizing the cellular reactions in the skin it can be said that consequent upon the penetration of larvae a considerable leucocytic infiltration of the skin takes place. After several successive infections the reaction takes place more quickly, and at 30 days after a series of twice-weekly infections neutrophils have begun to migrate to the area of penetration within one hour of application of the larvae. At 18 hours after infection considerable damage to the epidermis, probably caused by the larvae cutting the epidermal cells from their attachments, is evident. At this time neutrophil and

FIG. 3. Cellular reaction in the skin of a calf eighteen hours after penetration of larvae. (Haematoxylin-Eozin-Azure stain). ×714.

LEGEND.

| | | |
|---|---|---|
| n.l. | = | neutrophil leucocyte. |
| b.v. | = | blood vessel. |
| m.c. | = | mast cell. |
| p.c. | = | plasma cell. |
| e.l. | = | eosinophil leucocyte. |
| fi. | = | fibroblast. |
| p. | = | polyblasts. |
| e.c. | = | epidermal cells. |

eosinophil leucocytes infiltrate the skin in large numbers, but they appear to have no ability to check the progress of the larvae. Such phenomena can therefore be only a part of a mechanism of resistance, and evidently some other factor, probably humoral, is necessary before the cells in the skin are able to check the larvae. In order to investigate whether humoral factors are involved in infestation by *B. phlebotomum,* intradermal and precipitin tests were carried out.

*The sensitivity of the skin to extracts of the adult parasite*

An intradermal test was performed on Calves A and E. Calf A was infected twice weekly for two months; Calf E had re-received no infection when the first test was performed 30 days after the initial infection of Calf A. Calf A gave a positive reacton, while Calf E was negative. Later in the same day 200 larvae were applied to the skin of each calf and the area excised one hour later. From this time intradermal tests were carried out every 10 days up to 70 days after the first infection of Calf A. Calf E was given no more infections, but Calf A was exposed twice weekly for a further period of 30 days. Calf A gave positive reactions up to 60 days and then became negative. Calf E gave a positive reaction on the 50th day, but all other tests were negative.

Calf C, which received infections every other day for 35 days, was tested every seventh day with the following results: —

| *No. of days after first infection* | | *7* | *14* | *21* | *28* | *35* | |
|---|---|---|---|---|---|---|---|
| Result | — | — | + | + | + | + | Killed |

The reaction at 14 days was exceptionally vigorous. These results suggest that sensitization of the skin to extracts of the adult parasite follows repeated infection.

Calf A apparently became desensitized after three tests. The positive reaction of Calf E may have been due to sensitization either by its two previous tests or by its single application of larvae, although few parasites probably gained access to its body. On the other hand, it may have been a cross reaction due to the infestation with *Strongyloides.*

*Precipitin test*

This test was carried out on the serum of two calves, A and E, each of which was tested every 10 days for 70 days after the first infection of Calf A. While the serum of Calf E showed a negative reaction throughout the period of observation, the serum of Calf A formed a well-defined ring with the extract 40 days after initial

infection and continued to do so up to the end of the period of observation. It was tested again some months later and found to be negative.

It may be significant that the eggs of *Bunostomum phlebotomum* began to appear in the faeces of Calf A 56 days after its first infection, so that the serum gave the first positive reaction at about the time when adult parasites were probably beginning to attack the duodenal mucosa.

*Resistance to natural reinfection at pasture*

The following experiment was designed to test the effect of the periodic application of larvae on the resistance of calves to further entry of larvae and to discover whether resistance may result from parenteral administration of extract of *B. phlebotomum*. A plot of grassland about 20 yards square was fenced off and was burnt all over with a blowlamp to destroy ticks and nematode larvae. The following calves were placed on this pasture:—

Calves A and B, which had been infected twice weekly for two months. At the time of being placed on the pasture they were passing out 400 *B. phlebotomum* eggs per gramme of faeces.

Calf D, which was injected intravenously twice weekly with the extract of *B. phlebotomum* for two months before being placed on the pasture.

Calf E, which had received one infection of 200 larvae. The site of penetration was excised one hour later, so that very few larvae can have gained access. It had been subjected to five intradermal tests.

Calf F, which had received no infections. It was exposed to infection one month later than the rest.

The calves were placed on the pasture in April and received no further experimental treatment for four months. The intention was that Calves A and B should infect the pasture with eggs of *B. phlebotomum* in their faeces and that they and the other calves should become infected by the larvae which developed. The rains were so delayed, however, that it is likely that for the first two months conditions were unsuitable for penetration of the skin by the larvae.

Egg laying by the female hookworms begins within at least two months of infection (Sprent, 1946), so that by four months all the larvae applied to the skin of Calves A and B would be mature and any larval forms found in any of the carcases must have been picked up from the ground. Accordingly, after four months on this ground the calves were killed and the lungs, trachea, oesophagus and intestine were searched for the larvae and adults of *Bunostomum phlebotomum*. The findings were as follows:—

| | Adult females | Immature and larval forms |
|---|---|---|
| Calf A ... ... ... ... ... | 51 | 0 |
| Calf B ... ... ... ... ... | 181 | 0 |
| Calf D ... ... ... ... ... | 7 | 0 |
| Calf E ... ... ... ... ... | 50 | 150 |
| Calf F ... ... ... ... ... | 4 | 21 |

(Only 3 months at pasture)

It will be observed that the tissues of Calves A and B, which had received previous artificial infections, and Calf D, which had been subjected to intravenous injections of extract of *B. phlebotomum,* contained no larval or immature form of *B. phlebotomum.* The two Calves E and F, which had received no such treatment, both contained larvae. Calves A and B had had well over 10,000 larvae applied to the skin, and yet only light infestations were evident at the end of the experiment. Furthermore, at the time of being placed on the pasture both these calves were passing out 400 *B. phlebotomum* eggs per gramme of faeces. This figure suggests that Calf A was carrying a higher adult female population at that time than was later found at autopsy, in which case it not only resisted reinfection but had actually eliminated some of its parasites.

### *The cellular reaction in the intestine*

No evidence of inflammatory changes was observed in the lungs of artificially infected calves, but considerable reaction was seen in the intestine. Sections of the duodenum with the parasite still attached (Fig. 4) showed much destruction of the villi which appeared to have been sliced off at their bases, leaving the crypts of Lieberkühn embedded in a mass of inflammatory cells. The infiltration involved the lamina propria only, little change being observed outside the muscularis mucosae. Within the buccal capsule of the parasite lay a mass of cells, whose outlines were difficult to demarcate; they were evidently undergoing digestion, and this was most marked near the tip of the dorsal cone. Observations on the living worm showed that *B. phlebotomum* attacks the duodenal wall by scraping the surface into its mouth; the parasite draws its head dorsally and the two cutting plates at the ventral side of the buccal rim slice off the villi, so that the buccal capsule is filled with cells. From serial sections, it is evident that the four buccal lancets at the base of the buccal capsule prevent this plug of cells from blocking the oesophageal aperture and possibly tease the plug into shreds, facilitating the action of the secretion which flows from the dorsal cone. Disintegrated cells and pyknotic nuclei can be observed passing down the oesophagus of the parasite. The damage caused by these activities evidently initiates an intense cellular infiltration of the lamina propria. The contents of the buccal capsule are connected to the lamina propria by strands of connective tissue which from

the stretched appearance of the nuclei are under considerable tension. Scattered among these strands were masses of cells of various types, but there was little evidence of blood either in the parasite or in the surrounding tissues. The cellular elements observed in the lamina propria can be divided into granular and non-granular forms. The former include an enormous number of eosinophil leucocytes, especially evident in the vicinity of the attached parasite. Many of them near the mouth were disintegrated, the granules lying scattered among the neighbouring cells. Although most of them had lobulated nuclei, a number of eosinophil myelocytes were also observed. Neutrophil leucocytes were scarce and basophil leucocytes were not seen. A considerable number of irregularly shaped cells containing reddish purple granules and a pale oval nucleus were evident. (Fig. 5, basophil cells). Typical mast cells were not seen. Globular leucocytes with small darkly-staining oval nuclei and very large eosinophil globules in the cytoplasm were very common among the epithelial cells in the vicinity of the mouth of the parasite. Many of them had disintegrated, the globules being scattered among the contents of the buccal capsule. The on-granular series of cells consisted of a few small lymphocytes, a few typical monocytes and a very great number of cells which showed innumerable variations of nuclear and cytoplasmic structure from the large lymphocyte of the blood with deep blue scanty cytoplasm and large nucleus with large chromatin particles, through the monocyte form to the macrophage whose cytoplasm was more plentiful and less basophil and whose nucleus was more lightly staining than the lymphocyte. (Fig. 5. polyblasts). Large macrophages, probably of local origin, with grey vacuolated cytoplasm and large oval nuclei, and plasma cells, were also numerous in the lamina propria.

## DISCUSSION

While these observations are too few in number for firm conclusions to be drawn regarding the precise nature of the relationship between *Bunostomum phlebotomum* and the bovine host, they do suggest that, as a result of previous infection, the calf aquires a resistance to reinfection. It is probable that the defence mechanism consists partly of cellular and partly of humoral factors, as both the cellular reaction and the serum antibody content undergo marked changes consequent upon reinfection. It is possible to explain the findings of Mayhew (1940) on the grounds that the interaction of these two factors tends to keep the intestinal worm population at a constant level.

The cellular defence mechanism appears to be active in the skin at the site of the penetration of the larvae and in the intestine at the points of attachment of the adult parasites. It consists of an infiltration of neutrophil and eosinophil leucocytes in the skin and of eosinophil leucocytes and polyblast cells in the intestine. The first change noted during the course of infection was the increased

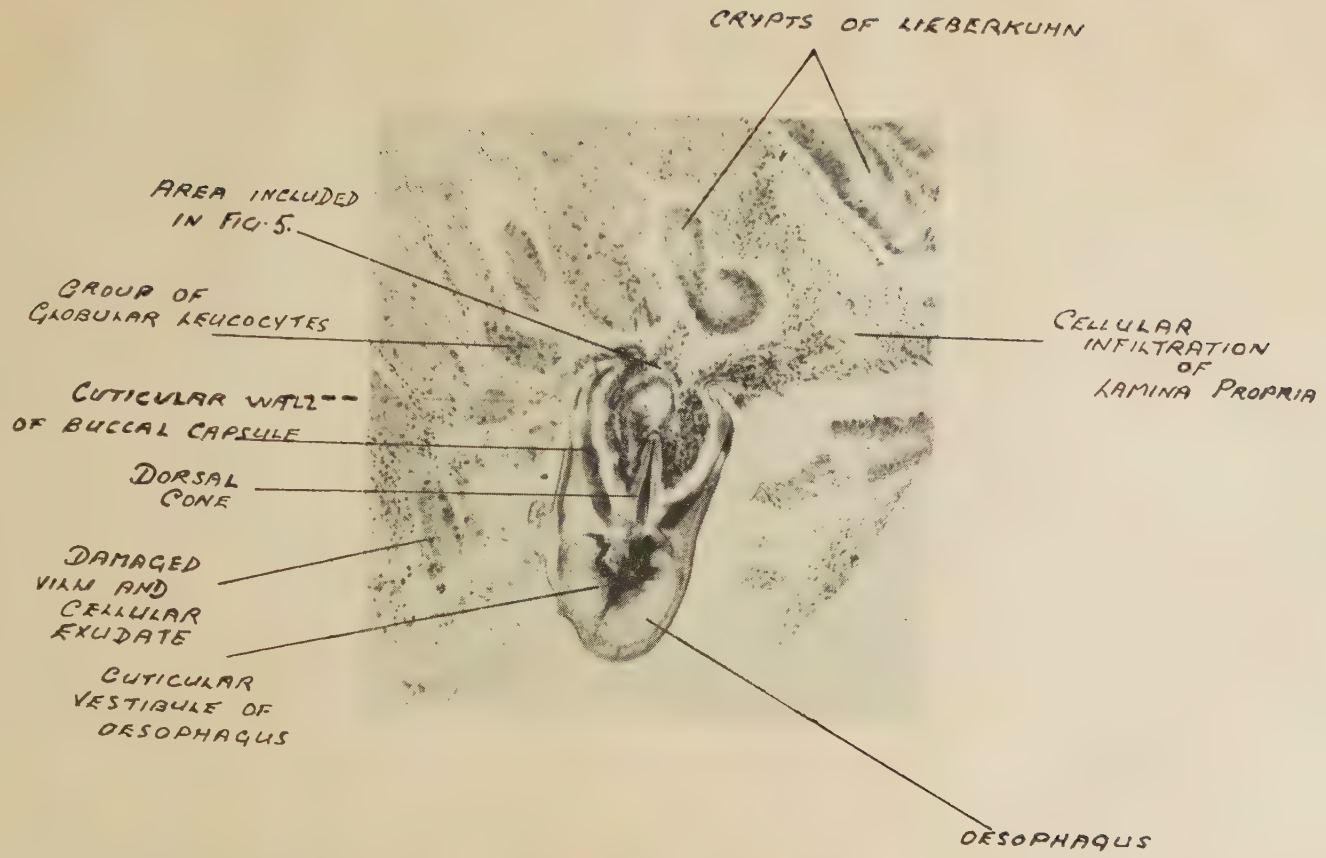


FIG. 4.—Section of duodenum of calf, showing the head of Bumostomum phlebotomum in site × 116.
(Haematoxylin - Eosin - Azure strain.)

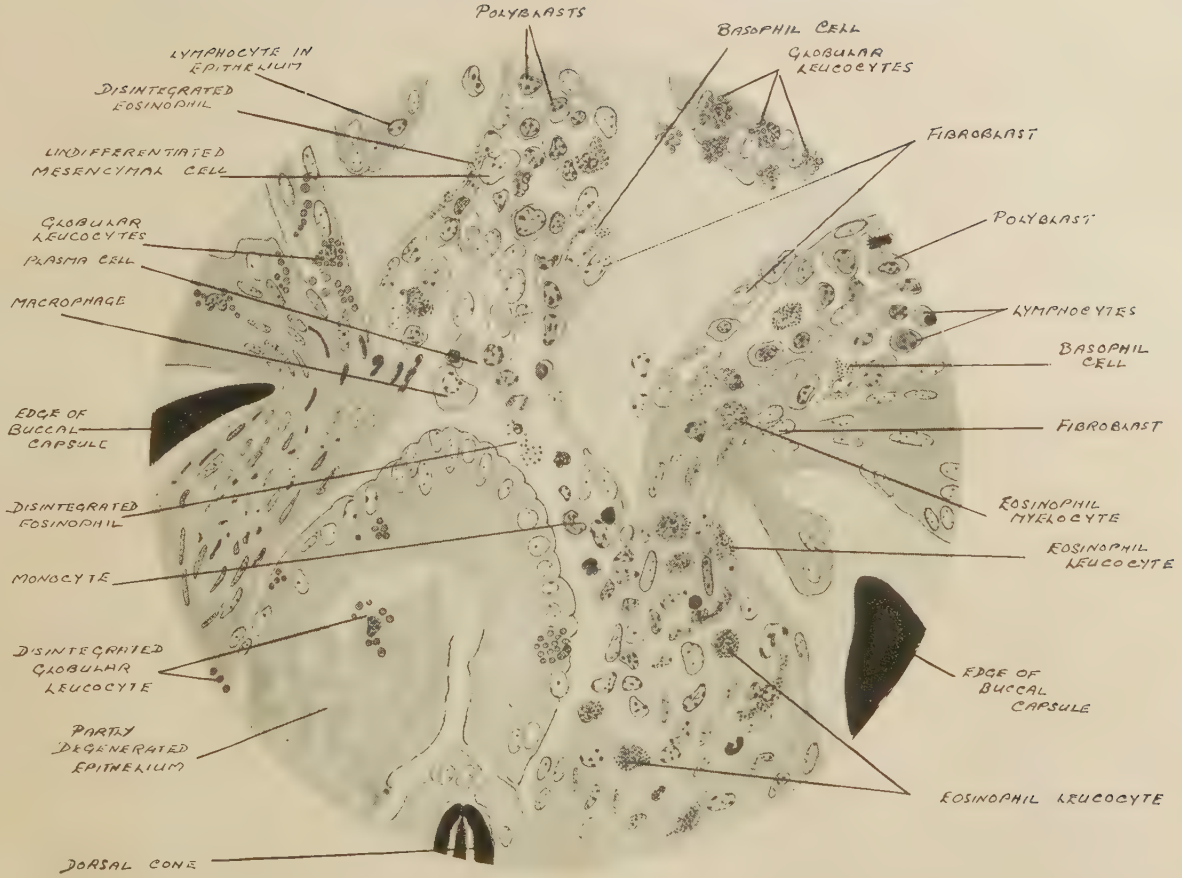


FIG. 5.—Drawing of the circular area demarcated in Fig. 4. ×714.
(Haematoxylin-Eosin-Azure Stain)

sensitivity of the skin to the penetration of larvae and to the injection of "extracts" of the adult parasite. This factor, involving more rapid and more extensive vascular reaction than occurs in normal skin, is probably of significance in the development of immunity and acts by facilitating diapedesis of leucocytes and the infiltration of antibodies. Antibodies were not, however, observed in the serum until later in the course of the infection *i.e.*, at about the time when adult parasites were becoming established in the intestine. It was shown above that intimate contact is effected between host tissue and adult parasite in the intestine, thus allowing for the passage of antigenic substances to the reticulo-endothelial system of the host .

It appears likely that cellular infiltration and increased vascular changes are not by themselves effective in combating the parasite, and it is possible, though not investigated, that resistance to reinfection is not fully developed until antibodies appear in the plasma and tissue fluids.

## SUMMARY

Periodic applications of the larvae of *Bunostomum phlebotomum* to the skin of calves brought into action a mechanism whereby the host reacted to their penetration by local vascular changes, neutrophilic infiltration and scab-formation. The speed of onset and extent of the reaction increased with successive reinfections. Nevertheless, a series of applications on alternate days over a period of 26 days failed to check the progress of larvae through the skin.

After a series of infections the skin of calves became sensitized to extracts of the adult parasite.

Forty days after the first application of larvae the serum of a calf infected twice weekly gave a positive ring test with an extract of the adult parasite.

At autopsy no larvae were found in the organs of calves which had been subjected to periodic infections of larvae or to periodic intravenous injections of hookworm extract, and subsequently exposed for four months on an infected pasture. Two calves not subjected to periodic infections both contained larvae.

Little macroscopic evidence of damage by the parasite or of host reaction was visible in the lungs, but the adult parasite produced a wound in the duodenum which permitted an intimate contact between host and parasite resulting in a considerable cellular infiltration in the area involved.

## REFERENCES

Mayhew, R. L. (1939). *Cornell Vet.*, **29**, 367; (1940). *Ibid.*, **30**, 495.
Otto, J. F., and Kerr, K. B. (1939). *Am. J. Hyg.*, **29**, 25.
Sheldon, A. J. (1937). *Ibid.*, **25**, 53.
Sprent, J. F. A. (1946). *Parasitol.* In Press.
Taliaferro, W. H. and Sarles, M. P. (1939). *J. infec. Dis.*, **64**, 157.

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* *Meyer, E., and Arnold, L., Amer. J. Digest. Dis., 5:418*

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